

CGT 4 **HIV** Cure

Cell & Gene Therapy for HIV Cure 2015

August 13-14, 2015

Fred Hutchinson Cancer Research Center
Seattle, Washington

cgt4hivcure2015.org



NOBEL LAUREATE STEM CELL EXPERT
DAVID BALTIMORE + PAULA CANNON

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THE FUTURE DEPENDS ON WHAT WE DO TODAY

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On behalf of the Scientific Organizing Committee, we are pleased to welcome you to the 2nd annual Conference on Cell & Gene Therapy for HIV Cure 2015. It is a privilege to host this year's event and we are proud to showcase the cutting-edge research in cell and gene therapy that has brought us closer than ever to finding a cure for HIV.

For the second year running, we are holding this conference at Fred Hutchinson Cancer Research Center, a fitting location for a conference established on the notion of finding a cure for HIV. A significant proportion of HIV-related gene therapy research builds directly off the success and refinement of bone marrow transplants, a technique developed by Fred Hutch's own E. Donnall Thomas.

Bone marrow transplantation has been used to effectively treat many diseases such as leukemia and lymphoma, and in 2007/2008, an allogenic bone marrow transplant was utilized to produce the first and only known cure of HIV. This single case gave gene therapy a tremendous boost, and legitimized the pursuit of an HIV cure.

Today's HIV treatments focus primarily on controlling the disease and mitigating its burden on those infected so that patients carrying the virus can live long productive lives. This form of therapy, while effective, has significant drawbacks with regard to cost, side effects, and lingering presence of the virus that is never fully eliminated.

Recent advancements in the gene therapy, transplantation and immunology fields have made HIV cure research a reality - a decade ago this goal was merely visionary. In the spirit of the Fred Hutch slogan, "Cures Start Here", we intend for this meeting to foster the collaborations and produce the science that will bring us all one step closer to reaching that ultimate goal.

Thank you all for your participation at this conference, and we hope you find the program engaging and informative in a way that will advance your own HIV research and advocacy.

Keith R. Jerome, MD, PhD

Co-PI, defeatHIV



Hans-Peter Kiem, MD

Co-PI, defeatHIV



SCHOLARSHIP RECIPIENTS

The Conference on Cell & Gene Therapy for HIV Cure is pleased to announce the 2015 scholarship recipients:

Biswajit Paul

Paul Munson

Mayumi Takahashi, PhD

Pavitra Roychoudhury, PhD

Sangeetha Satheesan, MSc

Nixon Niyonzima, MD, MSc.

Melanie Alvarado

Mesfin Gewe, PhD

Joumana Zeidan, PhD

Congratulations!

Galit Alter, PhD

Associate Professor of Medicine, Kristine and Bob Higgins MGH Research Scholar, Ragon Institute of MGH, MIT and Harvard

Paula Cannon, PhD

Associate Professor, Molecular Microbiology & Immunology, Pediatrics, Biochemistry & Molecular Biology, Keck School of Medicine, University of Southern California

defeatHIV Steering Committee

Keith R. Jerome, MD, PhD

Host, Conference on CGT4HIVCure2015

Co-PI, defeatHIV Martin Delaney Collaboratory

Member, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center

Professor and Head, Virology Division, Dept of Laboratory Medicine University of Washington

Hans-Peter Kiem, MD

Host, Conference on CGT4HIVCure2015

Co-PI, defeatHIV Martin Delaney Collaboratory

Member, Clinical Research Division, Affiliate Investigator, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center

Associate Head, Heme Malignancy Program, UW / Fred Hutch Cancer Consortium

Professor of Medicine / Adjunct Professor of Pathology, University of Washington School of Medicine

Ronald Mitsuyasu, MD

Professor, Department of Medicine, Director, Clinical AIDS Research and Education Center (CARE), University of California Los Angeles

defeatHIV Scientific Advisory Panel

Julie M Overbaugh, PhD

Member, Human Biology Division and Public Health Sciences Division, Fred Hutchinson Cancer Research Center

Erick Seelbach

HIV/AIDS Regional Resource Consultant, U.S. Department of Health and Human Services

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Geoff Symonds PhD

Chief Scientific Officer, Calimmune, Inc.

Jerome Zack, PhD

Professor, Microbiology, Immunology And Molecular Genetics, David Geffen School of Medicine, PI, UCLA CFAR, University of California Los Angeles

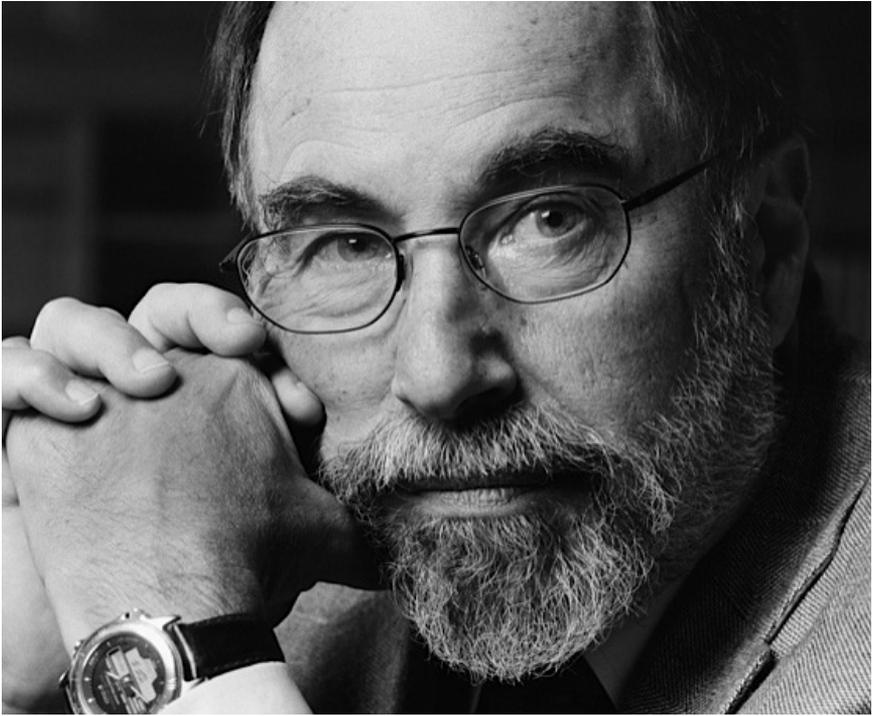
AGENDA | DAY 1:

Pelton Auditorium	7:00-8:00	Registration and Continental Breakfast
	SESSION 1	Opening Remarks & HIV Cure Overview
	8:00-8:15	Conference Welcome: Keith R. Jerome, MD, PhD; Hans-Peter Kiem, MD
	8:15-8:45	Pursuit of an HIV Cure: Jerome Zack, PhD
	SESSION 2	HCT for HIV Cure
	8:45-9:15	Richard Ambinder, MD, PhD
	9:15-9:35	Timothy Henrich, MD
	9:35-9:55	Leslie Kean, MD, PhD
	9:55-10:15	Break
	SESSION 3	Keynote
	10:15-10:30	Keynote Introduction: Lawrence Corey, MD
	10:30-11:30	Keynote Speaker: David Baltimore, PhD
	11:30-12:30	Lunch: Weintraub B-Suites
	12:30-1:00	Plenary Speaker: Matthew Porteus, MD, PhD
	SESSION 4	Gene Editing
	1:00-1:30	Paula Cannon, PhD
	1:30-1:45	Andrew Scharenberg, MD
	1:45-2:00	Daniel Stone, PhD
	2:00-2:15	Biswajit Paul
	2:15-2:30	Christopher Peterson, PhD
	2:30-2:45	Break
	SESSION 5	CAR T Cell Development for HIV Cure
	2:45-3:15	Lawrence Corey, MD
	3:15-3:30	Thor Wagner, MD
	3:30-3:45	Guillermo Romano-Ibarra
	3:45-4:00	Malika Hale
	4:00-4:15	Masakazu Kamata, PhD
	SESSION 6	Cure in the Community
4:15-4:30	Jessica Handibode, MBE	
4:30-4:45	Michele Andrasik, PhD	
Evening Activities		
5:00-6:30	Poster Session, Cocktails and Hors d'Oeuvres (SZE conference room)	
6:30-7:00	Shuttle from Fred Hutch to Space Needle	
7:00-10:00	Dinner Reception - Space Needle (downtown Seattle)	

Pelton Auditorium	7:30-8:00	Registration and Continental Breakfast
	8:00-8:30	Plenary Speaker: Dan Barouch, MD, PhD
	SESSION 7	Vaccines & Immunity
	8:30-9:00	James Mullins PhD
	9:00-9:15	Julie Overbaugh, PhD
	9:15-9:30	Paul Munson
	9:30-9:45	R. Keith Reeves, PhD
	9:45-10:00	Break
	10:00-10:30	Plenary Speaker: Michael Farzan, PhD
	SESSION 8	HIV Latency and Reservoirs
	10:30-11:00	Rafick-Pierre Sékaly, PhD
	11:00-11:30	Jerome Zack, PhD
	11:30-11:45	Matthew Strain, MD, PhD
	11:45-12:00	Joumana Zeidan, PhD
	12:00-1:00	Lunch: Weintraub B-Suites
	SESSION 9	Therapeutic Development & Gene Therapy
	1:00-1:30	Romas Geleziunas, PhD, Gilead
	1:30-2:00	Jeffrey Bartlett, PhD, Calimmune
2:00-2:15	Jennifer E. Adair, PhD	
2:15-2:30	Dong Sung An, MD, PhD	
2:30-3:00	Michael Holmes, PhD, Sangamo	
3:00 -	Closing Remarks: Keith R. Jerome, MD, PhD; Hans-Peter Kiem, MD	



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David Baltimore, PhD

President Emeritus;

Robert Andrews Millikan Professor of Biology

California Institute of Technology

1975 Nobel Prize in Physiology or Medicine

David Baltimore, former president of the California Institute of Technology (1997-2006) is President Emeritus and the Robert Andrews Millikan Professor of Biology. He is an accomplished researcher, educator, administrator and public advocate for science and engineering and is considered one of the world's most influential biologists. He received his BA in Chemistry from Swarthmore College in 1960 and a PhD in 1964 from Rockefeller University. Awarded the Nobel Prize in 1975 in Physiology or Medicine for his research into viral replication that provided the key to understanding the life cycle of retroviruses, Baltimore has profoundly influenced national science policy on such issues as recombinant DNA research and the AIDS epidemic.

His present research focuses on control of inflammatory and immune responses as well as on the use of gene therapy methods to treat HIV and cancer in a program called “Engineering Immunity”. In addition, he co-directs the Joint Center for Translational Medicine, an activity that joins Caltech and UCLA in a program to translate basic science discoveries into clinical realities.

Baltimore’s numerous honors include the 1999 National Medal of Science and 2000 Warren Alpert Foundation Prize. He is past-President and Chair of the American Association of the Advancement of Science and has published more than 680 peer-reviewed articles.

Engineering Immunity

D Baltimore

California Institute of Technology, Pasadena, CA

Our immune system does a fine job protecting us against a range of potential pathogens but it fails us in some key ways. Notable is the inability of most people’s immune system to conquer an HIV infection. Cancer too has been a difficult problem for the immune system to deal with. We have been examining ways that we can improve immune function by gene transfer into immunocytes or their precursors. Effective genes have been those that encode antigens, T cell receptors (TCRs), siRNA against CCR5 and antibodies. By setting up companies to exploit these opportunities we have seen them move into clinical trials. The more speculative technologies we have continued to develop in our own laboratory.

Two approaches are relevant to the HIV cures agenda. One is an siRNA against CCR5 being developed as an HIV therapy by Calimmune, Inc. It is in clinical trials but there are only anecdotal results. The second is antibodies to HIV gp120. These are not yet in humans but are in clinical development. The antibody program has prevention as its central goal and will be the major focus of this presentation.

We have shown that Adeno-Associated Virus (AAV) can be engineered to express full-length antibodies at levels that will protect mice carrying human CD4 cells from HIV infection. This methodology is a general platform that can be extended to influenza virus infection and malaria. It should be possible to extend it to provide a functional cure for an HIV infection.



Michael Farzan, PhD

Professor & Vice Chairman

*Department of Immunology and Microbial Sciences
Scripps Research Institute*

Dr. Michael Farzan is currently Professor and Vice Chairman, Department of Immunology and Microbial Science, at The Scripps Research Institute (TSRI) in Jupiter Florida. Dr. Farzan received his undergraduate degree in Government from Harvard College and his Ph.D. in Immunology from Harvard Medical School (HMS). He was appointed Assistant Professor at HMS in 2002, and left HMS as Professor of Microbiology and Immunobiology in 2012 to join TSRI.

Dr. Farzan's contributions include discovery of CCR5 sulfotyrosines and demonstration of their critical role in HIV-1 entry, discovery that the antigen-combining regions of HIV-1 neutralizing antibodies also incorporate functionally important sulfotyrosines, identification of the cellular receptors for the SARS coronavirus and for New World hemorrhagic fever arenaviruses, discovery of a family of restriction factors - the IFITM family - critical to the innate immune control of influenza A virus, and invention of eCD4-Ig, a very broad and potent HIV-1 entry inhibitor. His laboratory now focuses on the best ways to use gene-therapy vectors, antibodies, and engineered constructs like eCD4-Ig to prevent and treat HIV-1 infections.

AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges

M Gardner¹, L Kattenhorn², M Farzan¹

¹Department of Immunology and Microbial Science, The Scripps Research Institute, Jupiter, FL; ²Department of Microbiology, The New England Primate Research Center, Harvard Medical School, Southborough, MA

Background: Long-term *in vivo* expression of a broad and potent entry inhibitor could circumvent the need for a conventional vaccine for HIV-1. Adeno-associated virus (AAV) vectors can stably express HIV-1 broadly neutralizing antibodies (bNAbs). However even the best bNAbs neutralize 10-50% of HIV-1 isolates inefficiently (IC₈₀ > 5 µg/ml), suggesting that high concentrations of these antibodies would be necessary to achieve general protection. bNAbs in general are heavily hypermutated and in primate studies tend to elicit high levels of anti-idiotypic antibodies that interfere with their long-term expression.

Results: We show that eCD4-Ig, a fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide, binds avidly and cooperatively to the HIV-1 envelope glycoprotein (Env) and is more potent than the best bNAbs (geometric mean IC₅₀ < 0.05 µg/ml). Because eCD4-Ig binds only conserved regions of Env, it is also much broader than any bNAb. For example, eCD4-Ig efficiently neutralized 100% of a diverse panel of neutralization-resistant HIV-1, HIV-2, and SIV isolates, including a comprehensive set of isolates resistant to the CD4-binding site bNAbs VRC01, NIH45-46, and 3BNC117. Moreover, *in vitro* studies, eCD4-Ig-resistant viruses did not emerge under conditions where escape from CD4-Ig and NIH45-46 was readily observed. Rhesus macaques inoculated with an AAV vector stably expressed 17 to 77 µg/ml of fully functional rhesus eCD4-Ig for 54 weeks, and these macaques were protected from six escalating and infectious challenges with SHIV-AD8. The last of these was 16 times the 50% animal infectious dose of SHIV-AD8, administered more than a year after AAV inoculation. Rhesus eCD4-Ig was also markedly less immunogenic than rhesus forms of four well characterized bNAbs.

Conclusions: Our data suggest that AAV-delivered eCD4-Ig can function like an effective HIV-1 vaccine.



Dan Barouch, MD PhD

*Professor of Medicine, Harvard Medical School
Ragon Institute of MGH, MIT, and Harvard
Director, Center for Virology and Vaccine Research
Beth Israel Deaconess Medical Center*

Dr. Dan Barouch received his Ph.D. in immunology from Oxford University and his M.D. from Harvard Medical School. He is currently Professor of Medicine at Harvard Medical School, Chief of the Division of Vaccine Research at Beth Israel Deaconess Medical Center, and a member of the Steering Committee of the Ragon Institute of MGH, MIT, and Harvard. His laboratory focuses on studying the immunology and virology of

HIV-1 infection and developing novel vaccine strategies. His laboratory has explored a series of novel vaccine technologies, including adjuvanted DNA vaccines, poxvirus vectors, and alternative serotype adenovirus vectors in both preclinical and clinical studies. In particular, he has advanced a series of novel adenovirus vector-based HIV-1 vaccine candidates from concept and design to preclinical testing to phase 1 clinical trials that are currently underway in both the U.S. and sub-Saharan Africa. Dr. Barouch is board certified in Internal Medicine and Infectious Disease, and he is highly committed to teaching students, clinical fellows, research fellows, and junior faculty and to providing clinical care to patients with infectious diseases.

Broadly Neutralizing Antibodies for HIV-1 Eradication Strategies

DH Barouch

Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Ragon Institute of MGH, MIT, and Harvard, Boston, MA

We have previously shown that the broadly neutralizing monoclonal antibody PGT121 afforded substantial therapeutic efficacy in viremic, chronically SHIV-SF162P3-infected rhesus monkeys. In a recent study, we have also explored the efficacy of PGT121 in ART-suppressed, SHIV-SF162P3-infected monkeys. In addition, we recently observed that the viral reservoir is established very early following infection and prior to viremia. These data demonstrate new challenges facing HIV-1 eradication strategies and suggest novel approaches to target the viral reservoir.



Matthew Porteus, MD PhD

Associate Professor of Pediatrics

Stem Cell Transplantation and Regenerative Medicine, Hematology/Oncology, and Human Gene Therapy

Stanford University

Dr. Porteus received his MD and PhD degrees from Stanford, completed his clinical training in Pediatric Hematology/Oncology at Boston Children's Hospital and the Dana Farber Cancer Institute. He did his post-doctoral work with Dr. David Baltimore at CalTech where he began his studies on developing genome editing as a therapeutic tool for genetic diseases of the blood.

He currently practices on the Pediatric

Hematopoietic Stem Cell Transplant service at the Lucille Packard Children's Hospital at Stanford and runs a research lab in the Division of Stem Cell Transplantation and Regenerative Medicine, Hematology/Oncology and Human Gene Therapy that is focused on developing genome editing of stem cells as therapy for multiple genetic and non-genetic diseases. His research group has utilized many of the engineered nuclease platforms including ZFNs, TALENs, and CRISPR/Cas9 and has developed a number of assays that assess the functional toxicity of genome editing processes.

Nuclease Mediated Genome Editing to Create Stacked Genetic Resistance to HIV Infection

M Porteus

Stanford University, Palo Alto, CA

While the development of small molecule combination therapy (ART) for HIV has been a clear success story in biomedical research, the toxicity of the multi-drug regimen in some patients and the inability of this regimen to cure patients, means there remains for improvement in the medical management of this still devastating global pandemic. One of the hallmarks of HIV is its ability to mutate, evolve and escape single barriers to its lifecycle. Thus, our hypothesis has been that just as single monotherapy quickly led to the development of HIV resistance, that blocking the HIV lifecycle with single genetic blockades will also result in HIV escape and that instead multiple genetic blocks need to be created to confer long-term genetic resistance to HIV. Thus, the goal of our lab has been to develop an effective and safe approach to engineer hematopoietic stem and progenitor cells using genome editing such that the immune progeny of the modified cells will be genetically resistant to HIV through multiple mechanisms thereby creating a complex HIV resistant immune system to create a functional cure, if not sterilizing cure, for HIV.

SESSION 1 SPEAKERS

Jerome Zack, PhD INVITED SPEAKER

Professor, Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, PI, UCLA CFAR, University of California Los Angeles

HIV Cure Research: The Excitement Builds

J Zack

David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA

While current antiretroviral therapies are very effective at controlling HIV replication, they do not eliminate the virus. Rather, very stable viral reservoirs exist that will re-ignite active HIV infection upon cessation of therapy, thus HIV infected individuals must remain on antivirals for life. However enthusiasm is building for experimental approaches that may lead to elimination of viral reservoirs, and that may affect a cure, a “functional cure” or a durable remission in the absence of antiretrovirals. This presentation will discuss some of the important scientific advances that led to our current thinking about HIV cure research, and discuss some of the potential advantages and disadvantages of experimental approaches currently being tested in laboratories around the world.

Richard Ambinder, MD, PhD INVITED SPEAKER

Professor of Oncology, Director, Division of Hematologic Malignancies, Co-Director, Hematologic Malignancies, Johns Hopkins University

Allogeneic BMT with optimized antiretroviral therapy

RF Ambinder and C Durand

Johns Hopkins School of Medicine, Baltimore, MD

We have studied an antiretroviral regimen optimized for use in allogeneic bone marrow transplantation. The regimen includes antiretrovirals selected to minimize problematic drug-drug interactions and to maintain coverage during periods of vomiting or mucositis that might interfere with oral regimens. Primary endpoints included adverse events associated with enfuvirtide and maintenance of antiretroviral therapy through day 60. Donor chimerism and HIV infected cells per million (IUPM) by viral outgrowth assay were also measured. All have been treated with post-transplant cyclophosphamide based regimens. None have relapsed. With optimized antiretroviral therapy, it is feasible to maintain antiretrovirals but regimen changes are common due to drug interactions and organ dysfunction. Enfuvirtide is a well-tolerated alternative to oral therapies. Newer, non-oral and long acting antiretroviral agents may offer the same benefit. One patient self-discontinued antiretroviral therapy and developed an HIV meningoencephalitis raising concerns about retroviral rebound in patients whose immune systems are HIV-naïve.

SESSION 2 SPEAKERS

Timothy Henrich, MD INVITED SPEAKER

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

Stem Cell Transplantation, Innate Immunity and Graft-Versus-Residual HIV-1 Reservoirs

T Henrich

University of California San Francisco, San Francisco, CA

Allogeneic hematopoietic stem cell transplantation (HSCT) remains one of the few strategies that lead to significant, sustained reduction in viral reservoirs. Monophyletic HIV-1 rebound occurred after cessation of cART in the two “Boston Patients”, and one had undetectable HIV-1 8 months after treatment interruption suggesting the presence of very low numbers of infected cells. We also observed persistent, unique anti-HIV antibody signatures years after HSCT in the Boston and Berlin patients suggesting the persistence of long-lived plasma cells that survive chemotherapy and radiation. These antibody signatures change after viral rebound during the development of donor-derived cellular and humoral immune responses.

To better understand the mechanisms for the profound and sustained HIV-1 remission following allogeneic HSCT, we conducted in-depth analysis of HIV-1 reservoir size and immune responses in a total of 6 allogeneic HSCT participants, including one who received CCR5 Δ 32/ Δ 32 donor cells, and characterized the graft-versus-host (GVH) responses that permit donor cells to recognize and clear tumor cells or host cells of hematopoietic origin. We also developed an ex vivo assay to define the relationship between donor innate immune activation and function in samples from uninfected individuals that underwent allogeneic HSCT and to determine whether or not the beneficial GVH responses are specific for HIV-reactivated pre-HSCT CD4+ T cells.

Like the previously described Boston and Berlin patients, an additional subject who received CCR5 Δ 32/ Δ 32 donor cells had no detectable cell-associated HIV-1 DNA and RNA 3.4 months after HSCT in the setting of ART. HIV-1 DNA levels also markedly decreased in 2 other patients within 8 months following WT HSCT. Results from our ex vivo GVH assay demonstrated significant associations between NK cell (but not CD8+ T cell) responses and pre-HSCT CD4+ T cell viral reactivation. Our findings demonstrate that certain GVH responses following allogeneic HSCT may be HIV-1 specific and contribute to the profound and sustained reduction in viral reservoir size and prolonged ART-free remission following ART interruption.

Leslie Kean, MD PhD INVITED SPEAKER

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

Allogeneic Hematopoietic Stem Cell Transplantation and HIV Eradication: What Can we Learn from Translational Models of Graft-versus-Host Disease?

L Kean

Seattle Children's Hospital, Seattle, WA; University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA

It is now clear that the latent reservoir constitutes the major barrier to a sterilizing cure for HIV, and that the development of novel, paradigm-shifting approaches will likely be required for successful long-term viral control. Recently, hematopoietic stem cell transplantation (HCT) has offered the promise of a cure, but continues to contain significant challenges. The Berlin Patient, who received an allogeneic HCT (allo-HCT) with HIV-resistant cells, is the only patient cured of HIV. This finding has stimulated an intense investigation as to the components of HCT that contributed to his cure. These components include: (1) Pre-transplant conditioning; (2) Transplantation with allogeneic cells; (3) The development of GVHD; and (4) Transplant with HIV-resistant donor cells. While the Berlin Patient had all 4 components, it still remains unclear which of these components was necessary and/or sufficient for HIV eradication. Moreover, strategies by which to recapitulate the Berlin Patient's cure have not yet been developed. In order to study this problem, our laboratory has developed a Rhesus macaque model of allogeneic HCT and of GVHD. We have shown that in macaques, GVHD is caused by both CD4+ and CD8+ T cells, with the CD8+ T cells predominating during tissue infiltration. Target organs for CD8+ T cell infiltration include classic targets such as the liver, GI tract, skin and lung. In addition, our group has recently demonstrated that the brain is a target for CD8+ T cell infiltration during GVHD, an observation that may have specific relevance for HIV eradication, given the predicted need to eradicate CNS reservoirs as part of a sterilizing cure for HIV. Cells causing GVHD are characterized by their high proliferative index, their acquisition of high levels of cytotoxicity, and the accumulation of inflammasome-associated pathways of gene expression, as well as transcriptional programs controlling cell division and Th1/Th17 pathway choice. We are now combining HIV infection with both autologous and allogeneic HCT, in order to determine the impact that allo-activation, GVHD and T cell infiltration can make on HIV reservoir eradication.

SESSION 4 SPEAKERS

Paula Cannon, PhD INVITED SPEAKER

Associate Professor, Molecular Microbiology & Immunology, Pediatrics, Biochemistry & Molecular Biology, Keck School of Medicine University of Southern California

Targeted nucleases and HIV cure applications

C Exline¹, N Llewellyn¹, M Chateau¹, E Seclen¹, J Rathbun¹, C Chen¹, J Wang², M Holmes², P Cannon¹

¹University of Southern California, Los Angeles, CA; ²Sangamo Biosciences, Richmond, CA

Targeted nucleases such as ZFNs and CRISPR/Cas9 are providing exciting new capabilities for precise genetic modification of human cells, and are also finding several applications in HIV cure research. The gene disrupting capability of these reagents is being exploited in clinical trials using ZFNs targeting the CCR5 gene, in both T cells and HSC, and could also be used in the future to inactivate latent HIV genomes in reservoir cells. Recently, we have developed highly efficient methods to combine targeted nucleases with homologous donor sequences, presented as AAV genomes, and have thereby increased the capability of these reagents to site-specifically modify genes in HSC. We are currently exploring methods to deliver these reagents in vivo, and using humanized mouse models of HIV latency to help us towards these goals.

Andrew Scharenberg, MD INVITED SPEAKER

Professor, Department of Pediatrics, University of Washington, Adjunct Professor, Department of Immunology, University of Washington School of Medicine

Recombination-mediated gene editing in primary human T-cells

A Scharenberg

Seattle Children's Research Institute, Seattle, WA

Recent advances in gene editing nuclease platforms now allow the efficient targeting of any gene with a DNA double strand break. While many applications of gene editing can be achieved by allowing a targeted DNA double strand break to resolve via non-homologous end joining to disrupt or alter a gene's function, more complex genomic manipulations require efficient homology directed repair. We have identified AAV6 as an efficient means for delivery of homologous recombination templates to human T-cells, and demonstrate potential applications of the combined use of nuclease/AAV6 template delivery in engineering primary human T-cells for various forms of adoptive immunotherapy.

Daniel Stone, PhD ORAL ABSTRACT

Sr. Staff Scientist, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center

Emergence of treatment-resistant infectious HIV after genome-directed antiviral endonuclease therapy

D Stone¹, HS De Silva Feelixge¹, HL Pietz¹, P Roychoudhury¹, JT Schiffer^{1,2}, M Aubert¹, KR Jerome^{1,3}

¹Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA;

²Department of Medicine, University of Washington, Seattle, WA; ³Department of Laboratory Medicine, University of Washington, Seattle, WA

Background: Although HIV replication can be suppressed with antiviral drugs it is a major cause of morbidity and mortality worldwide. One potential approach to cure HIV is to use targeted endonucleases to prevent virus reactivation through the disruption of essential viral genes. However, a potential concern for endonuclease-based anti-HIV therapies is that treatment-resistant infectious virus might emerge.

Methods and Results: While testing the antiviral activity of HIV pol-specific zinc finger nucleases (ZFNs) in an in vitro model of HIV replication we identified a provirus encoding a treatment-resistant and infectious mutant NL4-3-ZFN2(+3) that was likely derived from a chance disruption within a ZFN target site in HIV reverse transcriptase (RT). Although ZFN-mediated disruption of target sites in protease, RT and integrase inhibited viral replication, a single amino acid insertion within the thumb domain of RT in NL4-3-ZFN2(+3) produced a virus that could replicate in TZM-bl cells and primary CD4+ T cells. NL4-3-ZFN2(+3) retained replicative capacity in primary CD4+ T cells, but it was as susceptible to treatment with antiretroviral RT inhibitors as wild type NL4-3. When additional ZFN-derived mutations were introduced into other target sites within the NL4-3-ZFN2(+3) RT or integrase domains, viral replication in TZM-bl or primary CD4+ T cells was inhibited.

Conclusions: Here we demonstrate for the first time the emergence of an endonuclease-resistant infectious virus following antiviral endonuclease therapy. Our observations suggest that caution should be exercised in the use of single endonuclease-based antiviral therapies for persistent viral infections, but that combinations of endonucleases can prevent or address the emergence of viral resistance.

Biswajit Paul ORAL ABSTRACT

Graduate Student, Molecular Cell Biology Graduate Program, University of Washington

Developing more efficient protective therapies against HIV: combining megaTAL nuclease-driven genome engineering with a resistance Cassette

B Paul^{1,2}, GS Romano-Ibarra³, DJ Rawlings³, A Astrakhan⁴, H-P Kiem².

¹Molecular Cell Biology Program, University of Washington, Seattle, WA; ²Fred Hutchinson Cancer Research Center, Seattle, WA; ³Seattle Children's Research Institute, Seattle, WA; ⁴Bluebird Bio, Inc.

Background: Human Immunodeficiency Virus (HIV) infection remains a substantial health problem worldwide. The human C-C chemokine receptor 5 (CCR5) gene, which encodes a co-receptor required for HIV entry into CD4+ T cells, is a promising alternative therapeutic target. Early clinical trials using CCR5-disrupting zinc finger nucleases in patients have demonstrated sustained functional control of HIV during antiretroviral treatment interruption. However, two limitations of current gene editing required to achieve therapeutic benefit remain unaddressed. These are (a) the need for higher levels of CCR5-disruption in long-term memory cells and (b) preferential selection of gene modified cells protected from subsequent infection during transplant.

Methods: The CCR5-targeting megaTAL is a novel nuclease architecture that combines a LAGLIDADG homing endonuclease scaffold with an eleven repeat transcription activator-like (TAL) effector array to achieve efficient site specific cleavage. We are coupling megaTAL nuclease treatment with drug selection in order to disrupt the CCR5 locus, and select modified CD4+ T-cells to achieve therapeutically relevant levels of HIV-protected cells. The mutant human dihydrofolate reductase (DHFR) construct renders cells resistant to lymphotoxic concentrations of the drug methotrexate (MTX) at 0.025uM. For optimal cell viability we deliver nucleases via mRNA and selection-constructs via adeno-associated virus (AAV).

Results: Electroporation with megaTAL mRNA demonstrated robust CCR5 disruption: 95% in GHOST-Hi5 cell lines and 70-90% in human CD4+ T-cells. Gene-modified human T-cells were transplanted into NOD/SCID/yc-null 'humanized' mice and subsequently challenged with HIV-1 infection. CCR5-null modified cells preferentially survived during active HIV infection in vivo (100 fold increase). Initially, the HIV plasma viremia was significantly lower in the nuclease-treated mice. However, the virus levels rebounded over time. We hope to address this by selectively transplanting cells that have been CCR5 disrupted and hence protected from any infection. In our chemoselection experiments, primary T-cells transduced with a Tyr-22-DHFR cassette at 15% efficiency showed an enrichment of >90% over 7 days in 0.025uM methotrexate. We contemporaneously achieved 60% AAV-mediated gene insertion of a GFP-reporter in primary T-cells long term.

Conclusions: The CCR5-megaTAL nuclease platform produces high levels of gene-modified primary human CD4+ T-cells and protects these cells from subsequent HIV infection in vivo.

Christopher Peterson, PhD ORAL ABSTRACT

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

Engraftment and Persistence of Zinc Finger Nuclease Gene-Edited Hematopoietic Stem Cells in a Nonhuman Primate Model of HIV/AIDS

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Background: Nuclease-mediated gene editing in hematopoietic stem cells (HSCs) holds great promise in the cure of HIV infection, but the feasibility and translatability of this approach to patients is unclear. To better evaluate the function of HSCs following gene editing, we have targeted the CCR5 locus and assessed engraftment following autologous transplant in a clinically relevant large animal model, the pigtailed macaque. CCR5 disruption in this model should directly protect against infection with simian/human immunodeficiency virus (SHIV). We are evaluating the extent to which CCR5-disrupted cell progeny engraft, and testing whether these cells impact SHIV viral reservoirs.

Methods: Zinc Finger Nucleases (ZFNs) are used to disrupt CCR5 in macaque HSCs; these stem cells and their progeny are subsequently measured ex vivo and in vivo. Animals are challenged with HIV enveloped SHIV, and suppressed by three-drug combination antiretroviral therapy (cART) following viral set point. Viral reservoir assays including 2LTR, single copy, and quantitative viral outgrowth (QVOA) are conducted on cART-treated animals before and after transplant.

Results: CCR5 targeting experiments yield up to 60% gene disruption in CD34+ cells ex vivo, translating to approximately 5% steady state bulk disruption in vivo. Gene disrupted cells demonstrate long-term, biallelic, multilineage engraftment in macaques. This approach is equally feasible in SHIV-naïve and in SHIV-infected, cART-suppressed animals. Early viral reservoir analyses suggest that persistent reservoir sites are seeded in our animals, and may be transiently impacted by the transplant procedure.

Conclusions: This is the first demonstration of successful long-term multilineage engraftment of ZFN-edited, CCR5-deleted HSCs in SHIV-naïve and SHIV-infected, cART-suppressed macaques. Our strategy results in robust levels of target gene disruption without impairing HSC engraftment or differentiation. Although CCR5-deleted cells can undergo SHIV-dependent positive selection, we are using gene targeting approaches to enrich for CCR5-edited cells without the need for ongoing viral replication. In these experiments, adenoassociated virus (AAV) is used to knock in a chemoselection marker at the ZFN-disrupted CCR5 locus in HSCs, which should allow for significant enrichment of infection-resistant cells in vivo. These results have important implications not only for HIV, but also other genetic diseases that can be treated by gene-editing of HSCs.

Lawrence Corey, MD INVITED SPEAKER

Member, Fred Hutchinson Cancer Research Center, Lawrence Corey Endowed Chair in Medical Virology, University of Washington

Immunologic Approaches to HIV Cure

L Corey

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

While retroviral latency is a sine qua non of cell lentiviral infections, there is evidence that HIV RNA is detected in many latently infected cells and that some HIV proteins/peptides may be expressed. The high rate of indirect killing of T cells during all phases of HIV suggests that HIV antigens may be displayed on such cells; even among persons with optimal clinical and virological control. Immunological approaches that would enhance recognition of these intermittent bursts of viral reactivation might lead to a functional eradication of HIV. The concept of a functional cure might be achieved through the development of HIV specific T cells that perform immune surveillance for infected cells expressing HIV proteins or peptides. Such effector T cells might provide both direct and indirect mechanisms of elimination of HIV reservoirs. Chimeric Antigen Receptor (CAR) T cells offer some potential advantages for such an approach. CAR T cells can be designed as “off the shelf” products; genetically engineered T cells that contain a combination of antigens that can be made or mixtures of such CARs to overcome antigenic diversity. The targets can be to conserved regions of the virus, thus obviating selection of immune escape variants. In addition, CAR T cells can be engineered to enter immunologically privileged sites.

This talk will discuss the potential of developing CAR T-cell therapy for long term benefit for HIV control and the potential to allow long term treatment interruption for patients with chronic HIV, and what obstacles may need to be overcome to achieve these goals.

Thor Wagner, MD INVITED SPEAKER

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

MegaTAL-based methods of CCR5 disruption to protect anti-HIV CAR+ lymphocytes from HIV Infection

TA Wagner^{1,3}, A Bernard,¹ M Hale², G Romano², I Khan¹, J Sahni², A Scharenberg^{2,3,4} and DJ Rawlings^{2,3,4}

¹Center for Global Infectious Disease Research and ²Center for Immunity and Immunotherapies and Program for Cell and Gene Therapy, Seattle Children's Research Institute, Departments of ³Pediatrics and ⁴Immunology, University of Washington, School of Medicine, Seattle, Washington,

Introduction: More than 30 million people are infected with HIV. Antiretroviral therapy (ART) dramatically decreases mortality, but HIV-infected individuals on ART have an increased risk of malignancies, cardiovascular disease, neurologic disease, and shortened life expectancy. Therefore a cure for HIV remains an important treatment goal. A previous Phase II randomized clinical trial of anti-HIV Chimeric antigen receptor (CAR)-expressing T-cells was partially effective. We hypothesize that a limitation of the previous strategy was that the anti-HIV CAR+ lymphocytes were susceptible to HIV infection.

Objective: Produce anti-HIV CAR expressing lymphocytes that are protected from HIV infection.

Methods: We designed novel anti-HIV CARs based on the scFV of a series of broadly neutralizing HIV antibodies. A CCR5 megaTAL nuclease (an engineered homing endonuclease and TALEN chimera) was used to disrupt CCR5 as a means of protecting lymphocytes from HIV infection. Two methods were used to produce CCR5-disrupted anti-HIV CAR+ lymphocytes. 1) Primary PBMC were transduced with anti-HIV CAR using a lentiviral (LV) vector, CAR+ were cells sorted, and then transfected with CCR5 megaTAL mRNA. 2) Alternatively, donor PBMC were transfected with CCR5 megaTAL mRNA followed by AAV delivery of the anti-HIV CAR with homology arms to induce homology directed recombination (HDR) of the anti-HIV CAR into the CCR5 locus. Cells that underwent HDR were sorted and expanded. CAR+ lymphocytes derived from each approach, were mixed with HIV-infected cell lines in the presence of ART or added to active HIV viral culture. The reduction in the number of HIV infected cells was assessed by flow cytometry and PCR. The reduction in replicating HIV was quantified by HIV capsid protein ELISA.

Results: LV delivery of anti-HIV CAR followed by sorting CAR+ cells and then CCR5 megaTAL transfection, resulted in CAR+ cells with up to 60% CCR5 disruption. HDR, followed by sorting and expansion resulted in approximately 40% CAR+ cells. Anti-HIV CAR+ lymphocytes upregulated cell surface CD137 and secreted IFN γ when mixed with HIV-infected target cells, killed a median of 75% (range 38-94%) of HIV-infected cells over 2-3 days, and reduced HIV DNA by approximately one log over 5 days. Beyond 72 hours of culture the anti-HIV CAR+ lymphocytes with CCR5 disruption resulted in much greater reduction in HIV than CAR+ lymphocytes without CCR5 disruption.

Conclusions: Different strategies are feasible to construct anti-HIV CAR+ T-cells that are protected from HIV infection. These strategy warrant further study using in vivo models of HIV latency.

Guillermo Romano-Ibarra ORAL ABSTRACT*Research Scientist, Seattle Children's Research Institute***Efficient targeted gene modification in primary human hematopoietic cells using co-delivery of nuclease mRNA and AAV donors**GS Romano-Ibarra¹, BD Sather¹, G Curinga¹, KM Sommer¹, M Hale¹, I Khan¹, S Singh¹, A Astrakhan², T Wagner¹, AM Scharenberg^{1,3}, DJ Rawlings^{1,3}¹Program for Cell and Gene Therapy, Seattle Children's Research Institute, Seattle, WA; ²BlueBird Bio Inc.; ³University of Washington, Seattle, WA

Current clinical HIV treatment strategies applying gene-editing technologies to disrupt the HIV co-receptor CCR5 via nuclease delivery are limited by inefficient bi-allelic disruption and the lack of a combinatorial approach to control HIV infection. The ability to target additional factors conferring HIV resistance to the CCR5 locus with efficient bi-allelic disruption has the potential to translate to improved clinical outcomes. Here we demonstrate a novel method in which RNA-based nuclease expression is paired with AAV-mediated delivery of a homologous gene-targeting donor template to achieve highly efficient targeted recombination in primary human T-cells. This method consistently achieves an average of 60% rates of homology directed recombination into the CCR5 locus in T-cells (>20 experiments with multiple donors); with up to 90% of the gene-modified population exhibiting bi-allelic modification. T-cells modified with this protocol maintain a diverse repertoire and engraft in immune deficient, NOD/SCID/ γ c^{-/-} (NSG) mice as efficiently as unmodified control T-cells. Using this method, we have integrated chimeric antigen receptors (CARs) into the CCR5 locus; and show that the resulting "targeted CAR" or tCAR T-cells exhibit anti-tumor activities indistinguishable from those generated using lentiviral random integration. Alternatively, we introduced the C46 HIV fusion inhibitor by targeted recombination generating T cell populations with very high-rates of bi-allelic CCR5 disruption paired with protection from HIV with CXCR4 co-receptor tropism. Next, as proof of principle, we utilized the CCR5 site as a 'safe harbor' locus for targeted gene addition of the human Wiskott-Aldrich syndrome (WAS) gene. Finally, this novel protocol was also successfully applied to adult human mobilized CD34⁺ cells, resulting in 10-20% homologous gene targeting, the first such report in adult human CD34⁺ cells at the CCR5 locus. Our results demonstrate that high-efficiency targeted integration is feasible in primary human cells, and highlight the potential of gene editing technologies to engineer CAR T-cells or other T cell products with myriad novel functional properties.

Malika Hale ORAL ABSTRACT

Research Scientist, Seattle Children's Research Institute, Seattle, WA

Targeted delivery of an anti-CD19CAR to the CCR5 locus in primary human T cells

M Hale¹, GS Romano-Ibarra¹, IF Khan¹, J Sahni¹, AM Scharenberg^{1,2}, DJ Rawlings^{1,2}

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Background: Lymphoma is the most common malignancy among HIV-infected persons in the United States. Cell therapies using patient or donor derived T cells engineered to express an anti-CD19 chimeric antigen receptor (CD19-CAR) continue to perform well in clinical trials for CD19+ malignancies, including diffuse large B cell and non-Hodgkin's lymphomas. Current protocols rely on lentiviral or gamma-retroviral delivery of the CAR construct, carrying an inherent risk of insertional mutagenesis, as well as heterogeneous expression of the CAR. Notably, these therapeutic T cells are also vulnerable to HIV infection, a particular cause for concern in extending this promising treatment to HIV patients. Here we show an efficient, alternative delivery method for the CD19-CAR, using homology-directed insertion of a gene cassette at the CCR5 locus, resulting in stable CAR expression and concomitant disruption of a critical co-receptor for HIV-1.

Methods: Primary human T cells were isolated from healthy donors and treated with an engineered megaTAL nuclease and donor template delivered via adeno-associated virus. This donor template consists of a gene cassette (MND-CD19CAR- t2A-BFP) between CCR5 homology arms. Seamless insertion of the gene cassette at CCR5 was confirmed by PCR and direct sequencing. Surface expression of the targeted CAR (tCAR) was detected by flow cytometry. Activity against CD19+ targets was measured by flow cytometry (CD137, CD107a, granzyme B) and ELISA (IFN- γ , IL-2).

Results: Primary human T cells treated with the megaTAL and CD19-CAR AAV maintained stable CAR expression (~15% by t2A-linked BFP and substantially higher using non-BFP linked constructs) and showed potent activity against CD19+ targets indistinguishable from controls transduced with an identical CD19-CAR construct delivered by lentivirus. In a mixed culture assay of CD19+ and CD19- target cells, the ratio of CD19+ to CD19- targets dropped to near zero in the presence of tCAR+ T cells ($p < .0001$, $n=3$).

Conclusion: Our method efficiently delivers the CD19-CAR construct to CCR5, resulting in sustained expression and CAR activity comparable to lentiviral delivery with the concomitant therapeutic benefit of CCR5 disruption. This approach is broadly translatable to other HIV therapeutics, including delivery of the C46 fusion inhibitor, and CARs targeting the latent HIV reservoir itself.

Masakazu Kamata, PhD ORAL ABSTRACT*Adjunct Assistant Professor, University of California Los Angeles***Ectopic expression of anti-HIV-1 shRNAs protects CD8+ T cells modified with CD4ζ CAR from HIV-1 infection and alleviates impairment of cell proliferation.**M Kamata¹, PY Kim¹, HL Ng², GE Ringpis³, E Kranz³, J Chan³, S O'Connor³, OO Yang^{2,3}, ISY Chen^{1,3},¹Division of Hematology-Oncology, UCLA David Geffen School of Medicine; ²Division of Infectious Diseases, UCLA David Geffen School of Medicine; ³Department of Microbiology, Immunology and Molecular Genetics, UCLA David Geffen School of Medicine

Chimeric antigen receptors (CARs) are artificially engineered receptors that confer a desired specificity to immune effector T cells. As an HIV-1-specific CAR, CD4ζ CAR has been extensively tested in vitro as well as in clinical trials. T cells modified with this CAR mediated highly potent anti-HIV-1 activities in vitro and were well-tolerated in vivo, but exerted limited effects on viral load and reservoir size due to poor survival and/or functionality of the transduced cells in patients. We hypothesize that ectopic expression of CD4ζ on CD8+ T cells renders them susceptible to HIV-1 infection, resulting in poor survival of those cells. To test this possibility, highly purified CD8+ T cells were genetically modified with a CD4ζ-encoding lentiviral vector and infected with HIV-1. CD8+ T cells were vulnerable to HIV-1 infection upon expression of CD4ζ as evidenced by elevated levels of p24Gag in cells and culture supernatants. Concurrently, the number of CD4ζ-modified CD8+ T cells was reduced relative to control cells upon HIV-1 infection. To protect these cells from HIV-1 infection, we coexpressed two anti-HIV-1 shRNAs previously developed by our group together with CD4ζ. This combination vector was able to suppress HIV-1 infection without impairing HIV-1-specific effector activities of CD4ζ. In addition, the number of CD4ζ-modified CD8+ T cells maintained similar levels to those of the control even under HIV-1 infection. Those results suggest that protecting CD4ζ-modified CD8+ T cells from HIV-1 infection is required for prolonged HIV-1-specific immune surveillance.

SESSION 6 SPEAKERS

Michele Andrasik, PhD INVITED SPEAKER

HIV Vaccine Trials Network Social Scientist, Fred Hutchinson Cancer Research Center

The importance of community engagement and participation in cure research

M Andrasik

HIV Vaccine Trials Network, Fred Hutchinson Cancer Research Center, Seattle, WA

Continuous community engagement and relationship building is paramount to the success of HIV Cure research. The utilization and implementation of Community-Based Participatory Research (CBPR) approaches are critical in the development and maintenance of reciprocal relationships between communities impacted by HIV/AIDS and research institutions. CBPR is a collaborative approach that involves the participation of community members more actively across the full spectrum of research from conception through the communication of results. CBPR efforts must occur early and often in the planning, design, implementation and interpretation of HIV Cure clinical trials. Effective CBPR approaches and strategies for developing these vital partnerships between community and academia will be revealed and compelling examples from the field will be presented. Actions to overcome challenges to academic/community partnerships will be explored.

Jessica Handibode, MBE INVITED SPEAKER

Program Coordinator AIDS Vaccine Advocacy Coalition (AVAC)

Thinking Beyond the CAB: Engaging Stakeholders in HIV Cure Research

J Handibode

AIDS Vaccine Advocacy Coalition, New York, NY

Early in the HIV/AIDS epidemic, activist groups engaged in science and research in new ways so they could speak knowledgably with pharmaceutical companies and government agencies about the research agenda and what communities wanted. They acted as translators between communities and researchers, establishing the model for community advisory boards (CAB). The CAB model remains important throughout HIV/AIDS and all other research, although it has also become clear that CABs may not fully represent all stakeholder groups that may impact research. The past ten years of HIV prevention research have demonstrated the importance of engaging a broad range of stakeholders early in the research process. Broad stakeholder engagement strengthens the ethical integrity of research by empowering stakeholder groups to be a part of the research process rather than subjects. It also supports the scientific aims by fostering trust through transparency in communities where research is being conducted. HIV cure research is at an exciting time as it seeks to raise and answer new scientific questions. These new questions raise difficult ethical challenges about trial design, risks versus rewards and participation. Engaging a broad set of stakeholders (e.g., industry partners, service providers, religious leaders and civil society) in HIV cure research is crucial to finding solutions to the challenging ethical questions.

James Mullins, PhD INVITED SPEAKER

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

The Conserved Elements (CE) approach to HIV vaccine design

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For many years HIV vaccine efforts focused on delivery systems and vectors, rather than on which viral proteins to include. Recently, however, greater effort has been placed on immunogen design, first exploring the use of central state and then variation inclusive immunogens. Most recently, we and others have proposed the use of subgenomic segments of viral proteins composed of only conserved regions of the viral proteome as immunogens in order to increase the breadth of strain recognition and redirect immune responses to the otherwise subdominant epitopes that have been associated with better immune control of infection. We engineered DNA-based immunogens encoding conserved elements (CE) of HIV-1 selected on the basis of stringent conservation, functional importance, broad HLA-coverage and association with viral control. DNA vectors were developed to express 7 collinearly arranged CE from p24gag found in >98% of HIV-1 group M sequences. These sequences were primarily located at p24 multimerization interfaces. By analogy to HIV, similar vectors were developed for SIV p27gag. Naïve and aviremic infected macaques were immunized with DNA encoding CE alone or in prime-boost regimens using DNA encoding the complete Gag. All CE DNA-vaccinated macaques developed robust CE-specific T cell and humoral immune responses. In contrast, upon repeated vaccination with HIV or SIV full-length gag DNA, only half of the animals developed T cell immunity targeting any of the CE. However, gag DNA vaccination significantly boosted the magnitude and breadth of preexisting CE T cell and antibody responses. CE immunogens also induced qualitatively different responses, with CE alone inducing multifunctional responses. Thus CE vaccines induce broad responses to vulnerable sites of the virus while avoiding "decoy" targets that divert effective T cell responses towards less protective epitopes. Our prime-boost approach provides a novel strategy to generating a prophylactic vaccine and to increase the magnitude and breadth of cellular and humoral immunity in infected hosts.

Julie Overbaugh, PhD INVITED SPEAKER

Member, Human Biology Division, Fred Hutchinson Cancer Research Center; Associate Professor, Department of Microbiology, University of Washington.

Defining the role of antibodies that mediate ADCC in protection from HIV infection and disease

C Milligan^{1,2}, J Mabuka^{1,2}, B Richardson¹, GJ Stewart², R Nduati³, J Overbaugh¹,

¹Fred Hutchinson Cancer Research Center, Seattle, WA; ²University of Washington, Seattle, WA; ³University of Nairobi, Nairobi, Kenya.

Antibody-dependent cellular cytotoxicity (ADCC) activity has been described as a potential immune correlate of protection from HIV infection and progression in macaques. The role of antibodies in protecting humans from HIV infection is less well defined although studies from the RV144 vaccine trial implicated ADCC antibodies in protection in exploratory analyses. We therefore evaluated the potential of ADCC antibodies to mediate protection in the setting of mother-infant transmission. Here, HIV-specific antibodies could act within the mother to reduce infectiousness or as passive protection in the infant, as infants acquire high levels of maternal antibodies at birth. Our studies show that in mothers at high risk of breastmilk HIV transmission, the level of ADCC antibodies in breastmilk is correlated with infant infection risk¹. In infants, passively acquired ADCC antibody levels were associated with clinical outcome in those who acquired HIV². In infants who were first detected HIV positive after birth, each 10% increase in passively acquired ADCC antibody activity was associated with a 49.1% reduction in the risk of mortality ($p=0.03$). The levels of HIV-specific IgG1, but not IgG3, were associated with survival in infected infants ($p=0.005$). Overall, these data suggest a therapeutic benefit of pre-existing HIV-specific ADCC antibodies. Thus, antibodies that mediate ADCC may be beneficial in reducing HIV infection and disease, including in the setting of therapy and cure.

1. Mabuka J et al PLoS Pathog. 8(6): e1002739, 2012.
2. Milligan C et al Cell Host & Microbe 17: 500-506, 2015

Paul Munson **ORAL ABSTRACT**

Doctoral Candidate, Department of Microbiology, University of Washington

Therapeutic conserved elements (CE) DNA vaccine increases T-cell responses against highly conserved viral sequences in the setting of pre-existing immunodominant responses induced by chronic viral infection.

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Background: We have previously shown that in SIV-infected rhesus macaques undergoing antiretroviral therapy (ART), therapeutic DNA immunization protected ~50% of animals from viral rebound after discontinuing ART. To improve this approach, we are investigating a novel conserved elements (CE) therapeutic DNA vaccine which consists exclusively of CE sequences. We hypothesize that a CE DNA vaccine will achieve a more profound functional cure by forcing immune escape mutations in regions of the virus that would have the greatest impact on viral fitness. A question that must first be addressed is whether immunization with a vaccine expressing conserved, but generally subdominant epitopes, can induce responses against CE in the setting of an immunodominant response induced by infection. To investigate this question, we compared immunogenicity of a CE DNA vaccine to a DNA vaccine expressing whole SIV Gag in rhesus macaques chronically infected with SHIV.

Methods: Two groups of rhesus macaques chronically infected with SHIV89.6P were immunized with either a traditional DNA vaccine expressing whole SIV Gag or an SIV CE DNA vaccine. An IFN- γ ELISpot assay was employed to map T cell responses induced in the blood and gut against the full SHIV proteome and the CE sequences. Intracellular cytokine staining was also used to assess functional quality of T cell responses directed against CE.

Results: Prior to immunization, both groups had similar responses to variable and immunodominant regions of Gag with little to no detectable responses to CE. Animals immunized with whole Gag exhibited no significant increase in responses against CE. In contrast, CE vaccinated animals developed a nearly ten-fold increase in IFN γ responses as well as a response skewed towards cytolytic effector function.

Conclusions: These results illustrate that a CE DNA vaccine was able to overcome immunodominant responses associated with a viral infection and re-direct the cellular response toward increased targeting of the subdominant conserved viral sequences when compared to a traditional full length Gag DNA vaccine. These results support the feasibility of developing a therapeutic CE DNA vaccine to induce a functional cure against AIDS.

R. Keith Reeves, PhD ORAL ABSTRACT

Assistant Professor of Medicine, Harvard Medical School, Beth Israel Deaconess Medical Center

NK cell memory in SIV- and SHIV-infected macaques

H Li¹, J Schafer¹, S Jost², DH Barouch¹, RK Reeves¹

¹Center for Virology and Vaccine Research, Harvard Medical School, Beth Israel Deaconess Medical Center; ²Ragon Institute of Massachusetts General Hospital, MIT, and Harvard.

Background: Natural killer (NK) cells provide rapid responses to viral infections and are typically considered to be nonspecific components of innate immunity. However, recent studies have shown that NK cells can also mediate antigen-specific memory in mice, but it remains unclear whether this phenomenon also exists in primates.

Methods: In this study we evaluated antigen-specific NK cells from a cohort of 18 rhesus macaques chronically infected with either SIVmac251 or SHIV-SF162P3, and 6 naïve controls. Using a novel flow cytometric assay we evaluated antigen-specific killing of autologous dendritic cells (DCs) by highly purified NK cells. Fluorochrome-labeled DCs were pulsed with peptides or intact SIV Gag or Env, and Ova-pulsed DCs served as intra-well controls. Purified NK cells were co-cultured with DCs at multiple E:T ratios and specific lysis was used as a functional determinate of antigen-specificity. A modified DC-NK co-culture assay was also used to identify CD107a+ and IFN-gamma+ antigen-specific NK cells by flow cytometry.

Results: Purified NK cells from SIV- and SHIV-infected animals were highly reactive to Gag- and Env-pulsed DCs at 10:1 NK:target ratios with a median specific lysis of 40%, indicating the presence of antigen-specific NK cells in both chronic infections. Peptide-scanning indicated dominant memory NK cell responses against SIV capsid (p27), but less frequently toward other regions of Gag. SIV-specific NK cells were rare in blood with dilution experiments estimating frequencies of < 1 in 1,000 circulating NK cells, but were up to two logs greater in frequency in liver, spleen, and lymph nodes, a potential site of HIV/SIV reservoir. Interestingly, NK cells in chronically-infected animals showed signs of exhaustion including upregulated Tim-3 and dysregulated cytotoxic functions.

Conclusions: Taken together, our data demonstrate the first evidence of NK cell memory in any primate species. Furthermore, antigen specific NK cell responses to SIV antigens are induced in primates following infection and accumulate in major sites of virus replication. The potent cytotoxicity and specificity of memory NK responses in primates suggest they could be critically important for control of HIV and SIV replication, and improved strategies to harness memory NK cells targeting infected and reservoir cells are warranted.

Rafick-Pierre Sékaly, PhD INVITED SPEAKER

Assistant Professor, Case Western Reserve University

FOXO3A and Interferons : a balancing act that controls reconstitution and HIV reservoir

RP Sekaly, K Ghneim, S Fourati, J Ahlers, M Cameron, C Shire, S Deeks, M Lederman

Case Western Reserve University, Cleveland OH, and University of California, San Francisco, San Francisco, CA

HIV eradication or a functional cure will become a possibility only when we will be able to control the persistence of the HIV reservoir and reconstitute the effector functions of the innate and adaptive immune system. We have used transcriptional profiling and unbiased systems biology approaches to identify pathways which could interfere with immune reconstitution in cART treated subjects; this approach was applied to define pathways that correlated with the magnitude of the inducible HIV reservoir in this cohort. Our work has allowed us to show that the balance between two transcription factors regulates immune reconstitution and the magnitude of the HIV reservoir. Foxo3A a transcriptional co-repressor that controls the expression of several pro-apoptotic and genes involved in the inhibition of cell cycles and IRF-7 the master switch gene of the innate and adaptive antiviral immune responses and a target gene of FOXO3A are responsible for regulating these two outcomes . We will present data that show the balance between FOXO3A and IRF-7 is regulated by perturbations in specific metabolic pathways. Targeting these metabolic pathways is an essential component of cell based and immune therapies.

Jerome Zack, PhD INVITED SPEAKER

Professor, Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, PI, UCLA CFAR, University of California Los Angeles

Novel PKC activators for HIV latency reversal

MD Marsden¹, X Wu¹, BA Loy², BA DeChristopher², AJ Schrier², CMR Kitchen¹, E Beans², D Fournogerakis², C Gauntlett², L Heumann², R Kramer², D Murray³, TW Chun³, PA Wender², JA Zack¹.

¹UCLA, Los Angeles, California, USA; ²Stanford University, Stanford, California, USA; ³National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA.

Background: Latently-infected CD4+ T cells represent a key barrier preventing the cure of HIV infection in patients treated with antiretroviral therapy (ART). One potential approach for eliminating this latent reservoir is to induce the virus to express new proteins, which would make the host cell susceptible to viral cytopathic effects, immune effector mechanisms, and other therapeutic approaches targeting viral proteins. For this strategy to be successful, safe and effective methods for activating latent HIV expression are needed. Protein kinase C (PKC) modulating agents such as bryostatin-1 and prostratin can stimulate expression of this latent HIV, potentially leading to its elimination. However, their use has been hampered by limited compound availability, and equally significantly by sub-optimal activity and in vivo tolerability. Our goals in the current study were therefore to design and synthesize improved latency activating compounds that function via the PKC signaling pathway.

Methods: Synthetic analogs of the natural PKC-modulating compounds prostratin and bryostatin were designed and synthesized. These were evaluated in several models for HIV latency, and in latently-infected cells obtained ex vivo from patients on suppressive ART. Compounds were also tested for bioactivity and acute toxicity in both immunocompetent and humanized bone marrow-liver-thymus (BLT) mice.

Results : Novel prostratin analogs were capable of potently activating HIV from latency at concentrations over 100-fold lower than the natural product. Several bryostatin analogs also activated HIV from latency more effectively than bryostatin-1. Notably, a selection of both prostratin and bryostatin analogs were highly bioactive (induced CD69 expression) in immunocompetent mice and showed improved tolerability compared with the natural parental compounds. Studies are currently in progress to determine if up-regulation of latent virus gene expression occurs in vivo in response to administration of synthetic PKC modulators.

Conclusions: Our data show that we can design synthetic PKC modulators that activate HIV from latency and have improved in vivo characteristics over the parental compounds. Due to their synthetic nature, these compounds can also be further optimized and "tuned for performance". Thus, these available and tunable bryostatin and prostratin analogs show promise as clinical adjuvants for HIV eradication.

Matthew Strain, MD PhD INVITED SPEAKER

Assistant Professor, University of California San Diego

Terminal-Dilution Assays Reveal Diverse Activation Responses within the Latent Reservoir

M Strain

University of California San Diego, San Diego, CA

Background: To test clinical interventions directed toward eradication of HIV, robust assays that precisely characterize persistent cellular reservoirs are needed. In this study, induction of HIV RNA by ex vivo activation was evaluated and compared with established assays based on quantitative viral outgrowth (QVOA) and total HIV DNA by droplet digital PCR (ddPCR) for total HIV DNA.

Methods: Peripheral blood CD4+ T cells harvested from 22 suppressed patients were serially diluted in an 8x12 format and activated with anti-CD3/CD28. Virus propagation was blocked with raltegravir. After 3 days of culture, induced cell-associated and cell-free HIV RNA were isolated with magnetic beads and quantified by RT-ddPCR for unspliced (gag) and multiply-spliced (ms) targets. Inducible cell frequencies were estimated by assuming single-hit Poisson kinetics.

Results: Among all assays considered, QVOA and inducible cell-free HIV RNA were the most similar ($r^2=0.63$, $p<0.001$). Transcriptionally-inducible cells were more frequent (median 20-fold and 3-fold for gag and ms HIV RNA), but were a poorer predictor of QVOA ($r^2[\text{gag}]=0.07$, $r^2[\text{ms}]=0.28$). The total number of induced HIV RNA copies per cell was heterogeneous, and cell-free ($r^2=0.42$, $p<0.02$) but not cell-associated HIV RNA ($r^2<0.26$, $p>0.10$) was correlated with QVOA results.

Conclusions: These data suggest that most released virus particles are replication competent. Viral transcripts can be induced in a much larger number of cells, but not in most cells harboring HIV DNA. Our results reinforce recent studies illustrating the diversity of replication-incompetent forms within the proviral reservoir. Proposed cure strategies may need to consider the effect of interventions on this spectrum of latent cells.

Joumana Zeidan, PhD ORAL ABSTRACT*Postdoctoral Research Fellow, Department of Pathology, Case Western Reserve University***Decay of the HIV Reservoir Post Autologous Transfer of ZFN CCR5-Modified CD4 T-cells (SB-728-T) Correlates with Generation of a Novel CD45-RO^{int} T Memory Stem Cell-Like Population**J Zeidan¹, G Lee², R Fromentin³, M Aid¹, J Lalezari⁴, R Mitsuyasu⁵, S Wang², W Tang², S Deeks⁶, D Ando², RP Sekaly¹¹Case Western Reserve University, Cleveland, OH; ²Sangamo Biosciences Inc, Richmond, CA, ³Centre hospitalier de l'Université de Montréal, Montreal, CA; ⁴Quest Clinical Research, San Francisco, CA; ⁵University of California Los Angeles, Los Angeles, CA; ⁶University of California San Francisco, San Francisco, CA.**Background:** Nine aviremic HIV+ subjects on ART received 10-30 billion SB-728-T cells. CCR5-modified cells expanded and persisted in PB up to 3.5 yrs post infusion (median = 2.1%), suggesting the presence of long lived CD4 populations, such as T memory stem cells (TSCM), within the product.**Methods:** TSCM phenotyping was performed on PBMCs pre- and post-infusion. Total HIV-DNA was measured using ddPCR. CD4 T-cell subsets were sorted in the product and post-infusion and the levels of CCR5 modification and integrated HIV-DNA were determined by qPCR. Gene array analysis was performed to determine pathways involved in long-term persistence.**Results:** We have previously identified a novel TSCM-like CD4 subset, characterized by co-expression of intermediate levels of CD45RA and CD45RO and high levels of CD95 and CD58, that expanded post-infusion and correlated with long-term CD4 reconstitution ($p=0.0279$). Gene array analysis suggested that persistence of this novel TSCM subset could be mediated by up-regulation of genes involved in self-renewal (Notch pathway) and metabolic pathways associated with cellular longevity (Fatty Acid Oxidation, OXPHOS, etc...). These cells originated from the product, as they were highly enriched in CCR5 modification ($23.2\% \pm 17.6$ modified alleles at 3yrs). Six out of 9 subjects displayed a significant decrease in levels of total HIV-DNA over time (range of decay from 0.5 to 3.6 log). Expansion of TSCM-like CD4 T-cells at month 6 correlated with the long-term decay of the HIV reservoir, which may be explained by their lower levels of integrated HIV-DNA (mean of 172 copies/1e6 cells vs 1133 and 2415 copies/1e6 for central (TCM) and effector (TEM) memory cells). Detection of up to 3% CCR5-modified TEM at 3yrs suggests that TSCM have the capacity to differentiate into other memory populations over time.**Conclusions:** A single infusion of CCR5-modified SB-728-T led to significant decay in the HIV reservoir. An HIV-resistant CD4 TSCM-like population can reduce the latent reservoir by selection and/or dilution, as well as by differentiating into effector memory cells and limiting reservoir replenishment. Our results suggest that targeting TSCM as a source of cells for genetic manipulation of lymphocytes can enhance efficacy of adoptive T-cell therapy. Acknowledgement: amFAR grant#108833-55-RGRL

Romas Geleziunas, PhD INVITED SPEAKER

Director, Clinical Virology, Gilead Sciences Inc.

Exploring Combination Therapies to Achieve ART-free HIV Remission

R Geleziunas

Gilead Sciences, Inc, Foster City, CA

We have been searching for strategies that activate production of AIDS viruses from persistent reservoirs and kill cells that produce viral proteins. Gilead currently has an ongoing clinical study testing a TLR7 agonist in patients with chronic HBV. We have recently demonstrated that dosing a TLR7 agonist in SIV+ rhesus monkeys on suppressive cART leads to transient plasma viremia which is accompanied by transient activation of CD8+ T-cells and NK cells. As a result of these in vivo findings, a clinical study was initiated to test a TLR7 agonist in HIV+ subjects on cART. Gilead has recently obtained an exclusive license from Theraclone Sciences to develop human broadly neutralizing monoclonal antibodies (bNABs) that interact with HIV envelope proteins. Once optimized, these bNABs will be tested to determine whether they can direct various immune effector cells to kill HIV-infected cells. Therapeutic strategies involving TLR7 agonists in combination with HIV bNABs merit thorough preclinical and clinical exploration for targeting reservoirs of HIV that persist during cART.

Jeffrey Bartlett, PhD INVITED SPEAKER*Senior Vice President, Calimmune Inc.***Engineering Cellular Resistance to HIV with a Dual Therapeutic Lentiviral Vector: Progress Toward the Clinic**

J Bartlett

Calimmune Inc. Tucson, AZ

Transplantation of CCR5^{-/-} hematopoietic stem cells has proven efficacious as a clinical approach for treatment of HIV-1 infection. We have developed a dual-combination anti-HIV-1 lentiviral vector (LVsh5/C46) that down-regulates CCR5 expression within transduced cells via RNAi, and inhibits HIV-1 fusion via cell surface expression of the fusion inhibitor, C46. This combination approach has two points of inhibition for R5-trophic HIV-1 and is also active against X4-trophic strains of HIV-1 that do not use CCR5 as a co-receptor

As a prelude to the clinic, safety was assessed in numerous in vitro models as well as GLP-like pharmacology/toxicology studies in NSG mice. In all cases LVsh5/C46 was shown to be safe and permit normal engraftment and differentiation of gene-modified cells. Furthermore, the humanized bone marrow, liver, thymus (BLT) mouse and pigtailed macaque models were used to characterize the ability of LVsh5/C46 to confer protection from HIV-1 or SHIV pathogenesis. In both models, animals transplanted with LVsh5/C46-modified CD34⁺ HSPC displayed no significant difference in hematopoietic engraftment and multi-lineage differentiation compared to controls. Both ex vivo and in vivo challenge of these models with appropriate HIV-1 or SHIV isolates demonstrated significant protection of CD4⁺ T-cells and reduced viral load within peripheral blood and lymphoid tissues. LVsh5/C46 gene-marking was confirmed stable post-transplantation and significantly increased following SHIV challenge of LVsh5/C46 treated pigtailed macaques. Positive selection of gene-marked cells was observed in all blood and tissue compartments examined. Finally, analysis of LVsh5/C46 integration sites in treated pigtailed macaques confirmed polyclonal engraftment of gene-marked cells. Following infection, a SHIV-resistant clonal repertoire was established in gene-marked T-cells, which maintained polyclonality. These findings revealed previously unobserved dynamics of T-cell selection in response to infection while offering the strongest preclinical evidence for safety and efficacy of LVsh5/C46.

A number of clinical trials utilizing LVsh5/C46 are now progressing, and if successful will advance the field in terms of how best to deliver and select for HIV-1 protected progeny. Ultimately, novel approaches will be necessary to bring gene-modified hematopoietic stem cell technology into a readily applicable strategy for the control of HIV-1 infection. An approach for how gene-modified hematopoietic cells may provide a viable therapy for cure of HIV-1 is provided.

Jennifer Adair, PhD ORAL ABSTRACT

Assistant Member, Clinical Research Division, Fred Hutchinson Cancer Research Center

Development of a Globally Portable Platform for Lentivirus Mediated Hematopoietic Stem Cell Gene Therapy to Treat HIV

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Anti-HIV lentivirus (LV)-mediated gene therapy of CD34+ hematopoietic stem and progenitor cells (HSPCs) is currently being tested in multiple clinical trials. Currently, state-of-the-art gene therapy requires ex vivo HSPC gene transfer in a dedicated Good Manufacturing Practices (GMP) facility, limiting treatment to wealthy countries. We developed a platform for efficient isolation and anti-HIV LV gene modification of bone marrow (BM) and mobilized peripheral blood CD34+ HSPCs in a closed system the Prodigy CliniMACS™. Seven programs were developed for this process: (1) hetastarch sedimentation to deplete red blood cells (RBCs), (2) labeling CD34+ cells in the RBC-depleted product, (3) immunomagnetic enrichment of CD34+ cells, (4) initial transduction (MOI = 20 IU/cell), (5) culture overnight, (6) second transduction (MOI = 20 IU/cell) and additional culture, and (7) harvest and formulation of the final product. Adding a pyrimidoindole derivative, UM729, facilitated efficient transduction of CD34+ HSPCs. The process took <30 hours from BM collection to cell product infusion with ≤3 hours hands-on time. Autologous, LV gene-modified CD34+ HSPCs from two nonhuman primates engrafted and supported hematopoietic repopulation after myeloablative total body irradiation. Cell doses were 27 x 10⁷ and 5.4 x 10⁷ CD34+ cells/kg body weight, respectively. Transduction efficiency in colony-forming units was 23% and 39%, respectively. We observed gene marking in peripheral blood granulocytes up to 40% and in lymphocytes up to 15% at >100 days after infusion. We then validated processing of human BM and mobilized apheresis products. We observed lower absolute CD34+ cell yields from BM products, however CD34+ cell yields from G-CSF mobilized peripheral blood apheresis products were equivalent or superior to non-automated methods. Transduction efficiency of >60% was demonstrated with a clinical anti-HIV LV currently in clinical trials. Resulting cell products from two donors were infused into immunocompromised mice and demonstrated engraftment of mature lymphocytes and granulocytes. These data demonstrate proof-of-principle of this system as a point-of-care strategy for ex vivo LV gene transfer into HSPCs, representing the first globally applicable advance in translation of anti-HIV HSPC gene therapy.

Dong Sung An, MD, PhD (Presenting on behalf of Saki Shimizu, PhD)

ORAL ABSTRACT

Associate Professor, UCLA School of Nursing; Director, Gene and Cellular Therapy Core, UCLA AIDS Institute

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

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Hematopoietic stem/progenitor cells (HSPC) based anti-HIV gene therapy hold a great hope for HIV cure. Current clinical studies have met with limited success largely due to the low efficiency of hematopoietic reconstitution with anti-HIV gene modified HSPC. We developed a novel chemoselection approach to overcome this limitation. To improve engraftment of anti-HIV gene modified HSPC, we investigated an in vivo selection strategy that exclusively employs 6-thioguanine (6-TG) for both pre conditioning and chemoselection of hypoxanthineguanine phosphoribosyltransferase (HPRT) down-regulated anti-HIV engineered HSPC. This approach is capable of enriching engraftment and long-term reconstitution of genetically engineered anti-HIV modified HSPC in vivo.

To provide 6TG resistance to gene modified cells, we have identified an HPRT short hairpin RNA (shRNA) that enables 6-TG mediated positive selection of lentiviral vector-transduced human T-cell lines, CD34+ cells and primary peripheral blood mononuclear cells (PBMC). Transduced cells were selected from 20 % to 90% within 7 days in vitro. Our in vivo engraftment experiment of CCR5shRNA and HPRT shRNA co-expressing vector modified human HSPC showed reconstitution of CCR5 down-regulated human T-cells in humanized BLT (huBLT) mice. Ex vivo isolated human splenocytes from the huBLT mice were positively selected by 6-TG. These results demonstrated that our newly developed HPRT shRNA could be combined with our CCR5shRNA in a lentiviral vector for positive selection. In addition to the positive selection, a novel feature of our HPRT knockdown strategy is a negative selection that is also possible to eliminate the HPRT knock down cells by using methotrexate (MTX). Thus, it can be developed as a safety procedure to eliminate gene modified HSPC in case of unexpected adverse effects observed in patients.

Our current results provide a RNA based novel chemoselection strategy that can be used for improving the engraftment of genetically protected cells for HSPC based anti-HIV gene therapy strategies.

Michael Holmes, PhD INVITED SPEAKER

Senior Director of Therapeutic Gene Modification, Sangamo Biosciences Inc.

Cross-Clade Inhibition of HIV on Primary Cells by CXCR4 or CCR5 Fused to the C34 Peptide from gp41 HR2

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HIV-1 entry into CD4+ T cells requires binding to CD4 and either the CCR5 (R5) or CXCR4 (X4) co-receptor. Thus, strategies that disable productive co-receptor (CoR) engagement should provide potent protection from HIV infection. Previously we described a 34 amino acid peptide from the C-terminal heptad repeat-2 domain of gp41 (C34) which, when fused to the amino terminus (NT) of either R5 or X4, inhibits HIV-1 infection in transformed cells in vitro. Moreover, our initial studies suggested that C34-R5 or C34-X4 fusions provided trans-dominant resistance to infection irrespective of viral tropism (i.e. either C34-R5 or C34-X4 could inhibit entry of R5, X4 or dual-tropic isolates).

Here we demonstrate that C34-R5 or C34-X4 expression by lentiviral transduction in primary CD4 T-cells from multiple donors results in almost complete inhibition (>98%) of HIV-1 infection based on intracellular p24 levels and RT activities. GFP-only and C34-CD4 expressing cells were infected at levels similar to untransduced T-cells. C34/CoR expression was >90% on Day 0 and stable during the 14 days of culture (>85%). Trans-dominant inhibition by C34-R5 or C34-X4 occurred for X4, R5 and dual-tropic primary isolates from clades B and A/E. Remarkably, when C34-CoR transduced and untransduced cells were mixed (1:4, respectively) and challenged with diverse HIV isolates, a condition that provides a more sustained exposure to HIV, selective enrichment of C34-CoR expressing cells occurred from the expected starting levels of ~25% up to 60% C34-CoR+ cells during viral replication. Lastly, PMA/ionomycin and anti-CD3/CD28 stimulation of C34-R5 and C34-X4 expressing T-cells resulted in the expected robust expansion of these T cells which produced levels of intracellular IFN γ , MIP-1b, TNF α , and IL-2 that were indistinguishable from untransduced cells. This novel method of engineering HIV-resistant, functional CD4 T-cells that can be expanded ex vivo and adoptively reinfused represents a promising and innovative approach with the potential to control HIV infection in humans.

Melanie Alvarado

Graduate Student, Department of Biological Sciences, University of Alaska Anchorage

Reactivation of latent HIV-1 by viral RNA PAMP is regulated by host RNA binding proteins

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Background: We are investigating innate antiviral immune responses to target and eradicate latent HIV reservoirs. Type I interferon pathways are triggered when cellular receptors bind and recognize pathogen-associated molecular patterns (PAMP) contained in viral RNA, activating transcription factors NF- κ B and IRF3/7 that induce antiviral interferon-stimulated genes (ISG), restricting virus replication. As NF- κ B also stimulates transcription of the HIV-1 LTR promoter, we hypothesized that viral RNA PAMP might induce a transcriptional cascade that enhances HIV expression in latently infected cells. We are also studying how novel host factors, including RNA-binding proteins and chromatin-modifying complexes, modulate HIV reactivation by viral RNA PAMP.

Methods: To test this hypothesis, we transfected J-Lat (H2) CD4+ reporter T cell lines containing an integrated HIV-1 sequence, 5' LTR promoter and a GFP reporter gene, with viral RNA PAMP: short chain poly-I:C, long chain poly-I:C (PIC-H), and Sendai virus RNA, in comparison to latency-reactivating drugs SAHA (a chromatin modifier) and prostratin (an NF- κ B and PKC activator). Transcription factors and novel host proteins thought to be involved were targeted by RNA interference (mRNA depletion) using siRNA.

Results: In J-Lat cells, HIV-1 transcription was activated by viral RNA PAMP, with PIC-H exhibiting a 10-fold increase. PIC-H concurrently induces IFN- β . We are also studying novel negative regulators of latent HIV reactivation. Targeting RNA binding proteins PKR, NF90, hnRNP A1, and RNase H2 with siRNA resulted in a 2- to 5-fold increase in HIV-1 transcription in cells transfected with PIC-H or Sendai virus RNA. Knockdown of PKR or NF90 also enhanced HIV-1 reporter expression by prostratin more than 2-fold. Interestingly, latency-reversing drugs prostratin and SAHA not only reactivate latent HIV, but significantly induced IFN- α , IFN- β , and interferon stimulated genes ISG56, MXA, and antiretroviral gene MXB.

Conclusions: Viral RNA PAMP can induce latent HIV-1 reactivation in a T cell reporter model, a process regulated by RNA-binding host factors. We are currently exploring the molecular mechanisms involved, and seeking to identify RNA species bound to host factors using next-generation sequencing. These innate antiviral immune pathways might be leveraged in new antiviral therapies to target and eradicate latent HIV reservoirs

Clarisse Benne, PhD

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

Transient differential expression of CD44 after TCR triggering in CD4 Central Memory cells as a marker of asymmetric fate of daughter cells

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Background: Asymmetric division has been suggested to play a role in the maintenance of long-term memory following T-cell activation while generating effector T cells. Previous studies showed that during T-Dendritic cell interaction, CD44 is asymmetrically distributed in favor of the proximal CD4 T daughter cells. CD44 is involved in different process such as migration, homing and activation. In CD4 T-cells, it stabilizes the immunological synapse, enhances TCR-induced proliferation and survival.

Hypothesis: We hypothesize that CD44 could discriminate the fate of daughter cells from CD4 central memory (CM) early after TCR engagement and that the distal CD44^{low} daughter cells from CM are long-term memory cells whereas proximal CD44^{high} daughter will become effector cells.

Method: Expression of CD44 was analyzed in sorted CD4 T-cells that were activated for 48hrs. CD4 CM and TM, CD44^{high} and low populations were identified and sorted. Wnt/Notch downstream targets were analyzed by Western blot and Microarray analysis were performed as well as proliferation assays.

Preliminary Results: Kinetic analysis of CD44 expression after TCR stimulation of CD4 CM and TM cells showed a transient differential distribution of CD44 (CD44^{high} and low) at 48h (N=10). CFSE dilution assays showed that the CD44^{low} subset presents a higher proliferation potential. Western blot analysis showed that the Wnt pathway is higher in CD44^{high} subsets as well as the apoptosis, unlike the Notch pathway that was lower in CD44^{high} cells.

Microarray data revealed that more than 1600 and 1900 genes were differentially expressed between CD4 CM CD44^{high} and low, and CD4 TM CD44^{high} and low, respectively (N=6, threshold ≥ 1.5 , $p \leq 0.05$). Preliminary analysis showed that signatures associated with Type II IFN, cell cycle or metabolic pathways are upregulated in the CD4 CM CD44^{high} subset.

Conclusion: The transient distribution of CD44 after TCR triggering could be used as a marker to identify and isolate long-term memory CD4 CM T-cells to compare their potential to the Stem cell-like memory T cells or to improve adoptive T-cell transfer therapies.

Janela McClure

Research Scientist, Department of Laboratory Medicine, University of Washington

A Simplified HIV-1 Quantitative Viral Outgrowth Assay that uses Cryopreserved Peripheral Blood Mononuclear Cells (PBMC) and FeederCell Stocks

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Background: New treatment strategies designed to eliminate HIV-1 cellular reservoirs require assays to reliably assess replication competent infectious virus. Our objective was to develop an HIV-1 viral outgrowth assay (VOA) that uses cryopreserved PBMC, essential for conducting multicenter, long-term clinical trials designed supporting the HIV-Cure agenda.

Methods: HIV-1 donor PBMC, normal peripheral blood monocytes and feeder PBMC were cryopreserved in liquid nitrogen. HIV-1 total or resting CD4+ cells were cultured at multiple replicates of 0.1, 0.5 or 1.0-million cells, stimulated with monocytes and anti-CD3 Ab for 3 days, then fed with CD8+-depleted phytohemagglutinin (PHA)-treated PBMC at 3 and 10 days. Culture supernatants were collected every 3-4 days and replenished with fresh media. HIV-1 p24 antigen (p24Ag) determined culture positivity. Supernatant was harvested for HIV-1 RNA and cells for 2LTR DNA (2LTR), RNA (c-RNA) and total nucleic acid (TNA).

Results: Replication-competent HIV-1 was detected at a median of 14 days of culture (range 7-32 days) from 14/14 antiretroviral treated (ART) aviremic (<40 RNA copies/mL of plasma) patients suppressed for a median of 5.33 years (IQR 3.34-8.93; range 0.58-14.23; CD4+ cell count, median, IQR, range: 487; 376-589, 223-858). Infectious units per million CD4+ cells (IUPM) ranged from 0.1 to 8.4 IUPM. Replicate assays on separate vials of cryopreserved cells returned similar results. Baseline levels of HIV-1 2LTR, c-RNA and TNA were not correlated with IUPM. HIV-1 RNA was detected in all VOA culture supernatants prior to p24Ag detection. Not all wells with high levels of HIV-1 RNA copies became positive for p24Ag, which suggests that non-infectious viral nucleic acid was released from the induced CD4+ cells. In selected experiments, VOA cultures were terminated at Day 14 and tested for p24Ag and 2LTR, an indication of viral propagation. All VOA positive culture wells that had detectable p24 in the supernatant also had 2LTR in the culture cells.

Conclusion: We have developed a convenient and reproducible HIV-1 VOA that detected infectious virus from cryopreserved PBMC. Thus far, none of the molecular markers of virus expression with the exception of 2LTR reliably predicted recovery of infectious HIV-1 as measured by production of p24Ag.

Nixon Niyonzima, MD, MSc

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Off-target cleavage activity of an HIV-specific engineered meganuclease

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Background: Mathematical models estimate that a four-log reduction in the size of the HIV viral reservoir can produce durable virologic remission. Towards the generation of an HIV curative therapy we acquired an engineered meganuclease (named “S20”) derived from I-Onu1 that recognizes and binds to a 22-nucleotide sequence in the pol gene of HIV. When S20 binds to its target HIV sequence, it generates a double strand DNA break that is repaired by error-prone non-homologous end joining (NHEJ), which can introduce inactivating mutations at the S20 target site. We have demonstrated mutagenesis of HIV pol using the S20 meganuclease. However, for the nuclease to be used in therapeutic applications, it should have minimal off-target cleavage activity. We therefore investigated the off-target cleavage activity associated with the S20 HIV-specific meganuclease.

Methods and Results: Using the online prediction program PROGNOS, we identified potential off-target cleavage sites associated with this engineered endonuclease. We examined the cleavage activity and specificity of the S20 meganuclease, using a yeast surface display system and FACS-based assay as previously described. A one-off cleavage analysis, using all 66 possible substitutions within the 22-nucleotide target sequence, demonstrated that the N-terminal domain of the enzyme displays considerable promiscuity in its corresponding recognition of the 5' half-site of the DNA target. We then selected the 30 closest matched off-target sites and performed a similar cleavage analysis against these targets. We observed significant cleavage of predicted off-target sites when the central four nucleotides (which are flanked 3 of 3 by the sites of DNA strand cleavage) were conserved, and the off-target sites primarily contained substitutions in the region recognized by the N-terminal domain of the enzyme. We also assessed off-target cleavage in eukaryotic cells expressing the S20 enzyme using the surveyor nuclease assay and clonal sequencing, and found that changes in the region that is bound by the C-terminus prevented cleavage by the enzyme.

Conclusion: An engineered HIV specific meganuclease displays significant off-target cleavage despite activity on target sequences. To meaningfully target HIV, a more specific endonuclease with minimal off-target cleavage activity will be required. We are currently working to improve the specificity of the S20 meganuclease.

Mark Pankau

Graduate Research Assistant, Human Biology Division, Fred Hutchinson Cancer Research Center

HIV reservoir size in HIV infected infants treated during acute infection

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Background: During acute human immunodeficiency virus (HIV) infection a viral reservoir is established when HIV integrates into resting CD4 T cells, and this reservoir persists throughout combination antiretroviral therapy (cART). In treated patients who have achieved complete viral suppression, activation of this reservoir of long-lived latently infected cells can lead to renewed HIV replication after treatment is discontinued. In adults, earlier cART initiation and time to viral suppression have been shown to decrease viral reservoirs to levels similar to elite controllers; however, data on the relationship between early ART and viral reservoir size in infants is more limited. We validated a droplet digital PCR (ddPCR) assay using cross-subtype pol primers to quantify levels of total HIV DNA in samples with known quantities of HIV DNA. Using previously validated serial dilutions of HIV proviruses we show that ddPCR can accurately quantify HIV copy number from 100 copies down to 2 copies of HIV DNA in a human genomic DNA background of approximately 100,000 cells. We then used this ddPCR pol assay to quantify HIV DNA in samples from a cohort of Kenyan infants treated with cART prior to 1 year of age (median age cART initiation: 4.9 months). We limited our analysis to 39 infants who maintained viral suppression throughout 2 years on cART, defined as having at most most 1 viral load above 1,000 copies/mL. After 24 months of cART, HIV DNA levels were detectable in 38 out of 39 infants, with a median of 233 HIV DNA copies/1e6 PBMCs (IQR: 90-494). Infants who started cART before 3 months of age had lower levels of HIV DNA than those treated after 3 months: median 77 HIV copies/1e6 PBMCs vs 248 HIV copies/1e6 PBMCs, respectively (p=0.09). Our data is consistent with studies suggesting that earlier cART during acute HIV infection may limit viral reservoir size in infants.

Pavitra Roychoudhury, PhD

Postdoctoral Research Fellow, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center

Predictive pharmacodynamics model of DNA cleavage enzyme delivery for curative HIV gene therapy

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Background: Our group is developing a gene therapy approach for the cure of HIV, relying on efficient delivery and expression of DNA cleavage enzymes within infected cells. These enzymes are engineered to bind and mutate specific target sequences within latent HIV genomes, rendering the virus replication incompetent. Delivery is achieved with viral vectors that contain the enzymes as a transgene payload. Delivery is assessed by quantifying expression of a fluorescent reporter gene using flow cytometry (FCM). Here we present a mechanistic model that predicts quantitative transgene expression in target cells as a function of vector dosage. We fit the model to FCM data from three experiments aimed at optimizing transgene delivery to HIV-permissive CD4+ memory T-cells in culture using self-complementary adeno-associated virus (scAAV) vectors. We identify that delivery follows a sigmoidal dose-response relationship and that the level of saturation of gene expression depends on the serotype, promoter and experimental conditions. Delivery saturates at a maximum of ~30 vector genome copies per cell (vg/cell) when using unpurified scAAV stocks or ~9 vg/cell with purified scAAV. Of the different serotypes and promoters, scAAV1 vectors with the EF1 alpha short promoter have the lowest particle to cell ratio required for saturation. In co-transduction experiments, we find that cells that express one reporter gene at high levels have a greater than random chance of expressing the other reporter. In order to obtain more than 95% co-transduction, vectors need to be added at ratios of at least 50000. For a given serotype and promoter, the model accurately predicts the minimum dose needed to obtain a desired level of transduction and provides a method of dose selection that can be applied to the cure of HIV as well as other gene therapy applications.

Mayumi Takahashi, PhD

Post-Doctoral Research Fellow, Department of Molecular and Cellular Biology, Beckman Research Institute, City of Hope

Selection of CCR7 Targeting Aptamers for HIV Latency

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HIV can establish stably integrated, non-productive state of infection known as HIV latency. Because latently infected cells have low or no viral gene expression and cannot be distinguished from uninfected cells, HIV latency is a major obstacle for eradication of the virus. Although combinatorial anti-retroviral therapy (cART) against HIV infection can suppress the level of plasma virus in patients below detection limit, interruption of therapy could cause a rebound in viremia. The development of cell-type specific approaches either reactivating latency or purging reservoirs is likely to improve therapeutic specificity and decrease side effects that are associated with current antiviral therapies. We have previously shown the specific aptamers to deliver siRNAs into target cells. Therefore we reasoned that aptamers can function as a cell-type-specific delivery agent.

CCR7 is a well-known marker of central memory CD4⁺ T cells which is known as major HIV latent reservoir, thus we hypothesize that HIV reservoir can be targeted using CCR7 aptamer. For aptamer selection, we have combined a well-established “Live-cell-based SELEX (Systematic Evolution of Ligands by Exponential enrichment)” strategy with high throughput sequencing (HTS) technology and bioinformatics analysis to identify new 2'-fluoropyrimidine modified RNA aptamers directed to human CCR7. In addition, we employed digital droplet PCR (ddPCR) for PCR amplification step in each selection cycle to minimize the amplification bias between sequences. As a result, significant molecular enrichment was observed after 5 round of selection, suggesting that specific sequences against CCR7 have been successfully enriched. Furthermore, the molecular diversity of ddPCR selection was significantly larger than conventional open PCR selection. The individual sequences were classified into nine groups based on the alignments of aptamer sequences and secondary structures from which we selected synthesized 18 unique sequences for further characterization. These aptamers were labeled with Cy-3 fluorescent dye, and bindings and cellular uptakes were determined by flow cytometry. Cell-type specific binding was observed for aptamers on CCR7^{high} T cell line (H9 cells, 48-77%), while only minimum binding was observed on CCR7^{low} T cell line (Jurkat cells, <16%).

Sangeetha Satheesan, MSc

Graduate Student, Department of Molecular Biology, City of Hope

Development of humanized NSG mice to evaluate HIV persistence

S Satheesan

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A current challenge to the efforts to eliminate HIV-1 from infected individuals is the establishment of persistent long-lived HIV infected cells in the blood and various anatomic compartments. The resting memory CD4+ T cells are the best characterized HIV-1 reservoir and they can be detected in blood, lymph node and the gut. HIV can also persist in the mononuclear phagocytes in different anatomic compartments such as central nervous system, lung, liver, lymphoid tissue etc. A more complete understanding of the role of these cells and anatomical locations would greatly enhance attempts to design and test viral purging strategies. We have utilized NOD/SCID/ γ c null (NSG) mice for our studies as NSG newborn mice can be successfully reconstituted with human lymphoid and myeloerythroid components following fetal-liver derived hematopoietic stem cell injection. Our results demonstrate that humanized NSG mice support production of human cell types permissive to HIV-1 infection. We also assessed ability of these mice to sustain long-term infection in vivo by infecting them with X4-tropic HIV-1 and viral infection was assessed by PCR and CD4+ T cell levels in peripheral blood were quantified by flow cytometry. Our results demonstrate viremia in humanized NSG mice and that HIV-1 infection leads to CD4+ T cell depletion in peripheral blood, thus mimicking the key aspects of HIV-1 pathogenesis. The NSG mice with demonstrable HIV infection were treated for six to ten weeks with combinatorial antiretroviral therapy composed of drugs that block new infections, but not drugs that inhibit the viral production of infected cells. The persistence of HIV during the antiretroviral treatment is due to the latently infected resting CD4+ T cell population in the post integration phase of infection. As the first step to identify latency in the infected resting CD4+ T cells, we have established the presence of the resting cell population in the naïve humanized NSG model. The viral reservoir has been evaluated by culture-based assays (viral outgrowth assay) and PCR-based assays (digital droplet PCR).

Chen Hsu-Yu, MSc

Graduate Student, Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California

Examining the potential of heparin binding-deficient AAV6 vectors for gene delivery to human hematopoietic stem cells

HY Chen, M Chateau, C Wang, E Seclen, C Lim, P Cannon
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Adeno-associated virus vectors with capsids of serotype 6 (AAV6) can robustly transduce human hematopoietic stem cells (HSC), making them good gene therapy tools to manipulate these cells in anti-HIV gene therapy approaches. In particular, we have found that AAV6 vectors can be used in combination with zinc finger nuclease mRNAs to deliver homologous DNA donor templates to HSC and thereby promote high levels of homology-directed gene editing. We are currently exploring whether the *in vitro* tropism of AAV6 vectors for HSC is also preserved *in vivo*, using systemic or localized injection into humanized mice. As one approach to increase the efficiency of this process we are engineering AAV6 to reduce *in vivo* tropism by removing receptor binding elements that are not required for HSC entry. For example, AAV6 is known to use both heparan sulfate and sialic acid as receptors, and competition studies with heparin suggested that this was component not necessary for HSC binding. We have therefore introduced a single point mutation, K531E, which has been reported to reduce the ability of AAV6 to bind to heparan sulfate and are examining the impact of this mutation on HSC transduction, both *in vitro* and *in vivo*. In summary, we aim to develop an improved AAV vector to achieve specific gene editing in HSCs *in vivo*.

Mesfin Gewe, PhD

Post-Doctoral Research Fellow, Basic Sciences Division, Fred Hutchinson Cancer Research Center

Spectrum of somatic hypermutations in anti-HIV-1 neutralizing antibodies and the implication on antibody function

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Sequence diversity, ability to evade immune detection and the establishment of latent reservoirs present a formidable challenge to the development of protective vaccines against human immunodeficiency virus type 1 (HIV-1). A potential vaccine development approach, attempting to circumvent these challenges, aims at designing vaccine immunogens based on infection elicited and clinically isolated broadly neutralizing antibodies (bNAbs). However, such antibodies characteristically exhibit extensive mutations following antibody maturation. Understanding the importance of these high-frequency mutations in overall antibody function is imperative to the success of a structure-based vaccine design. Here we report a biochemical and structural characterization for the antibody maturation of infection elicited bNAbs b12. Using a panel of point reversions on mature antibody and the progressive incorporation of mutations on b12 germline precursor antibody, we examined the relevance of individual amino acid mutations in antibody function. Biophysical analysis of b12 point mutant interactions with gp120 monomers indicate the importance of cooperative contributions by individual mutations in attaining broadly neutralizing properties of mature b12. In addition, a potential mechanism in attaining increased epitope binding affinity is through antibody conformational rearrangement and rigidification of antibody variable domains. Our structural analysis of antibody light chain variable regions revealed the absence of an apparent effect on the global structure of antibody light chain variable domains following antibody maturation while local structural rearrangements manifested as conformational samplings were observed. Given our results, we conclude a successful structure-based vaccine design that utilizes b12-like bNAbs as templates need to develop a scheme that enables extensive antibody maturation.

Erick Seelbach

HIV/AIDS Regional Resource Consultant, U.S. Department of Health and Human Services, defeatHIV and CFAR Community Advisory Boards

Bringing Community to Cure

ET Seelbach¹, L Sylla¹, AJ Kumar¹, T Andrus², M Louella²

¹defeatHIV Martin Delaney Collaboratory, Community Advisory Board; ²defeatHIV Martin Delaney Collaboratory, Fred Hutchinson Cancer Research Center, Seattle, WA

Background: The defeatHIV Community Advisory Board (dHCAB) is an effective catalyst for community engagement and feedback between HIV cure researchers and the community, as well as for collaboration among different local HIV-related CABs, such as those for CFAR, ACTU, and HVTU. The dHCAB is the local CAB for the defeatHIV Martin Delaney Collaboratory based at Fred Hutchinson Cancer Research Center in Seattle, WA, one of three NIH-supported cure collaboratories.

Methods: The dHCAB has employed multiple strategies for community engagement, including: community forums with HIV cure research leaders; opportunities to meet Timothy Ray Brown; providing multi-cultural food and music at education events; holding community events at different community organizations and venues; visiting agencies to engage them in cure research; tabling at events such as the annual AIDS walk and pride festivals, theater productions, etc; webinars; active use of social media, including Facebook, Twitter and Youtube. Those on the dHCAB have had the opportunity to attend and present at scientific meetings and to provide input to researchers on protocols, informed consent documents, and recruitment.

Results: More than 1000 community members have participated in dHCAB events and we have reached thousands more through outreach. The dHCAB currently consists of 12 members. Members range in age from high school to senior citizens and include women, gay and straight men, individuals in recovery, newly diagnosed individuals and long term survivors, African-Americans, Asians, Latinos, and Native Americans. Programs typically bring in dozens to hundreds of participants. The defeatHIV investigators consider the dHCAB a valuable asset and partner and have taken feedback to heart. We have learned about community concerns and provided meaningful pathways for education and dialogue.

Conclusions: With creativity, perseverance and respect it is possible to engage the community and researchers in meaningful ways that will advance HIV cure research.

The Collaboratory of AIDS Researchers for Eradication (CARE)

Results of a community needs assessment and pilot test of a novel HIV cure research training curriculum

K. Dubé¹, J. Taylor², R. Jefferys³, M. Sharp⁴, S. Wakefield⁵, J. Handibode⁶

¹Collaboratory of AIDS Researchers for Eradication (CARE), The University of North Carolina at Chapel Hill (UNC-CH), Chapel Hill, NC, USA; ²Collaboratory of AIDS Researchers for Eradication (CARE) Community Advisory Board, Palm Spring, CA, USA ³Treatment Action Group (TAG), New York City, NY, USA, ⁴National Martin Delaney Collaboratory (MDC) Community Advisory Board (CAB), San Francisco, CA, USA, ⁵HIV Vaccine Trial Network (HVTN), Seattle, WA, USA, ⁶AIDS Vaccine Advocacy Coalition (AVAC), New York City, NY, USA

Background: HIV 'cure' or remission research demands scientific literacy for participants and researchers regarding participation. An international collaboration of advocates, NGOs and researchers has created a curriculum to facilitate understanding and scientific cooperation. Iterative evaluation with intended audiences is being conducted with community groups during curriculum development.

Methods: During the period of August -October 2014, participants attending HIV cure research training sessions completed an online HIV cure research literacy needs assessment. The needs assessment questionnaire fed into the development of a comprehensive HIV cure research literacy curriculum. Individuals who completed the needs assessment questionnaire were invited to participate in pilots of three different curriculum modules. Participants evaluated the content and usefulness of modules, assessed HIV cure research 'literacy' levels and sought real-time feedback from participants to develop and refine subsequent training modules.

Results: Of the n = 42 respondents, 40 (95.2%) found the curriculum module very useful and 1 (2.3%) found it somewhat useful, as opposed to not very useful or not at all useful (1 answer missing). Most participants found the module very easy (32/42; 76.2%) or somewhat easy (12/42; 28.6%) to understand, as opposed to not very easy or not at all easy. In-person talks or forums (27/42; 64.2%) were the preferred method of learning, compared with webinars/conference calls (5/42; 11.9%) or self-paced web-based learning programs (8/42; 19.0%). Participants preferred training topics by the community included therapeutic vaccines (31/42; 73.8%), participation in HIV cure research (27/42; 64.2%) and ethical issues (26/42; 61.9% of participants).

Conclusions: The needs assessment of the curriculum effort showed the usefulness of the training and desires to increase community 'literacy' around HIV cure research. Based on the results of the pilot tests and needs assessment questionnaire, 15 curriculum modules were developed and scaled-up by scientific and community liaisons. International training program implementation includes PowerPoint teaching sets, pre-/post-test assessments, online and in-person presentations as well as participatory activities. The curriculum can strengthen community capacity to participate in and make decisions around HIV cure research. However, greater efforts to systematically roll out the literacy tool is needed in diverse community populations.

Delaney AIDS Research Enterprise Community Advisory Board (DARE).**The DARE CAB: A Model for Community Involvement in Basic HIV Research**

M Agosto, D Evans, R Jeffreys, B Lein, C Martinez, S Muchnick, S Schwarze, M Sichone, A Thompson.

The NIH funded several awards under the Martin Delaney Collaboratory: Toward an HIV-1 Cure (U 19). Martin Delaney was a prominent activist involved in policy and research reforms from the earliest days of the AIDS epidemic until his death in 2009. He advocated strongly for funding focused research efforts to cure HIV infection and AIDS. In the spirit of Martin's legacy, it is essential that community be involved in all levels of decision making in the new collaboratories, but few models exist for community interaction with basic, as opposed to clinical, HIV research. Increasingly the role of community has been understood and embraced in the clinical research realm: providing input to trial design, informed consent, recruitment and education. It is less clear what the role of community can and should be in basic HIV research endeavors. The Delaney AIDS Research Enterprise (DARE) to Cure HIV's Community Advisory Board (CAB) represents a model for community involvement in basic HIV research and, we believe, is useful to present in establishing best practices in this arena.

We describe the DARE CAB's structure and goals and how it interacts with DARE's projects and scientific leadership. We discuss successes, challenges and lessons learned. We summarize its accomplishments and current initiatives, including activist and educational projects.

Ruth Sedlak, PhD

Research Scientist, Molecular Virology Lab, Department of Laboratory Medicine, University of Washington

Digital detection of endonuclease mediated gene disruption in the HIV provirus

RH Sedlak¹, S Liang¹, N Niyonzima², HS De Silva Feelixge², P Roychoudhury², ND Weber^{2†}, S Boissel⁵, AM Scharenberg, A Cheng³, A Magaret^{2,3}, R Bumgarner⁴, D Stone², KR Jerome^{*1,2}

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Genome editing by designer nucleases is a rapidly evolving technology utilized in a highly diverse set of research fields, including HIV cure efforts. Among all fields, the T7 endonuclease mismatch cleavage assay, or Surveyor assay, is the most commonly used tool to assess genomic editing by designer nucleases. This assay, while relatively easy to perform, provides only a semi-quantitative measure of mutation efficiency that lacks sensitivity and accuracy. We demonstrate a simple droplet digital PCR assay that quickly quantitates a range of indel mutations with detection as low as 0.02% mutant in a wild type background and precision ($\leq 6\%CV$) and accuracy superior to either mismatch cleavage assay or clonal sequencing when compared to next-generation sequencing. The mutation efficiency of a megaTAL that targets HIV pol was assessed by ddPCR and mutations rates greater than 30% were achieved on a plasmid-derived HIV target. The precision and simplicity of this assay will facilitate comparison of gene editing approaches and their optimization, accelerating progress in this rapidly-moving field.



defeatHIV, the Delaney Cell and Genome Engineering Initiative

Founded in 2011 and led by Drs. Keith Jerome and Hans-Peter Kiem at the Fred Hutchinson Cancer Research Center, the defeatHIV Martin Delaney Collaboratory is a consortium of scientific investigators and clinicians from both public and private research organizations who are committed to finding a cure for HIV. We are supported through a program sponsored by the National Institutes of Health and the National Institute of Allergy and Infectious Disease in honor of AIDS activist Martin Delaney. This program, called the Martin Delaney Collaboratory: Towards and HIV-1 Cure, focuses on providing support for HIV research strategies that are curative and fosters partnerships between public and private research organizations. defeatHIV is one of only three funded Martin Delaney Collaboratories, which also include the Collaboratory of AIDS Researchers for Eradication (CARE) based at the University of North Carolina at Chapel Hill, and the Delaney AIDS Research Enterprise (DARE) at the University of California, San Francisco.

As a Martin Delaney Collaboratory program, we are inspired to re-examine existing approaches in the fight against HIV/AIDS and to focus our energies on developing innovative and novel strategies to abrogate the spread of this debilitating disease. Our core technologies utilize the latest cell and genome engineering approaches to create HIV-resistant cells for transplant, and to develop rare-cutting endonucleases that may seek out and destroy HIV in its hiding places throughout the body.

It is our mission to leverage the knowledge, expertise and resources of the consortium to generate a realistic promising pathway toward an HIV cure.

defeatHIV.org



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Fred Hutch occupies 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. More than 2,825 people work for Fred Hutch, including more than 200 scientific faculty and more than 570 pre-doctoral and post-doctoral researchers and other scientific staff.

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.

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CFAR – Center for AIDS Research

University of Washington
Fred Hutchinson Cancer Research Center
Center for Infectious Disease Research
Seattle Children's
Cure Scientific Working Group

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

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

www.depts.washington.edu/cfar

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

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

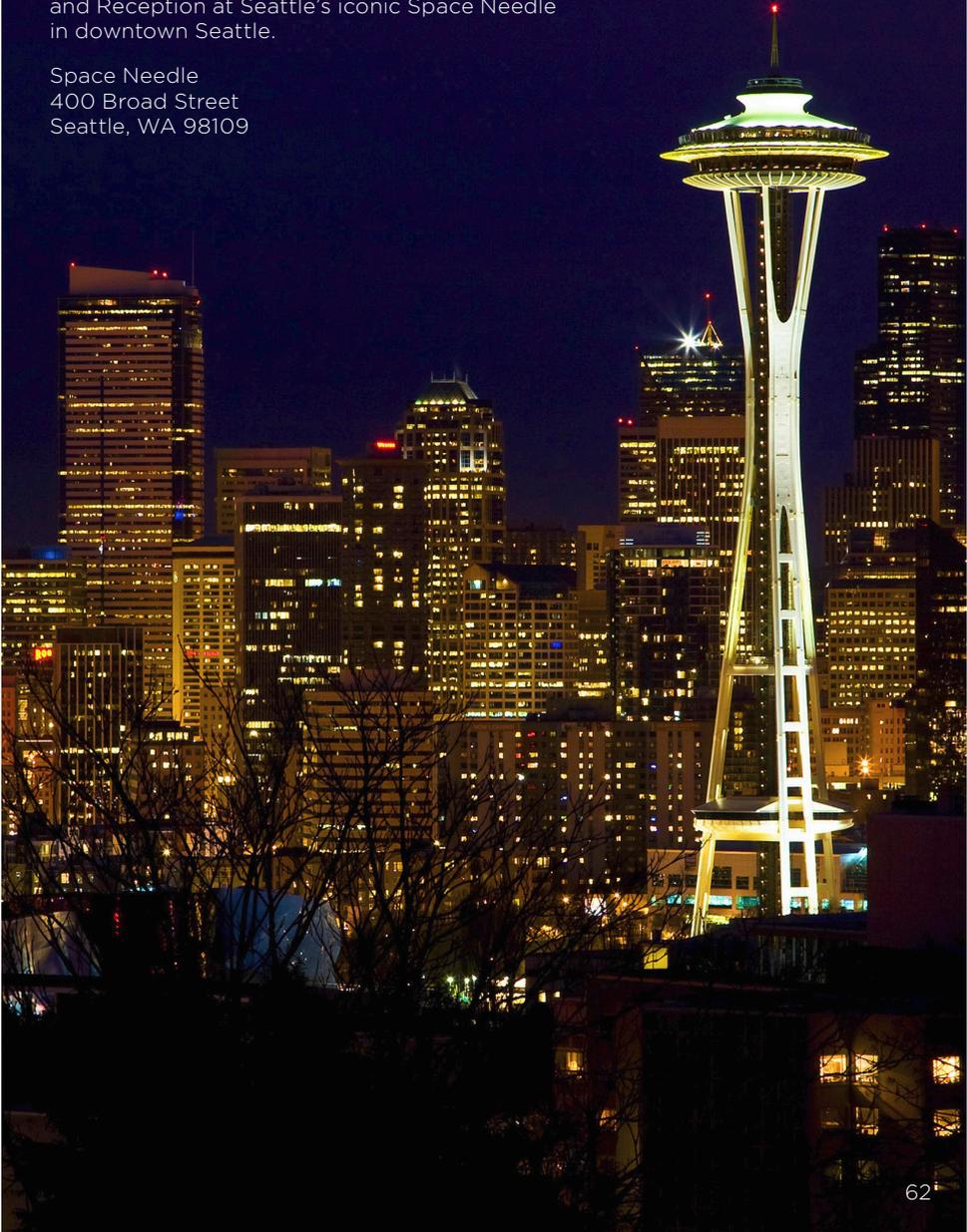
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The conference is pleased to be supporting the attendance and participation of Community Advisory Board members from the Martin Delaney Collaboratories of CARE, DARE, and defeatHIV.

Matt Sharp

*National Martin Delaney Collaboratory
CAB Coordinator*

David Palm, MSc

CARE CAB

Jeff Taylor

CARE CAB

Andy Kaytes

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Martha Sichone-Cameron

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