IN MEMORIAM:

This conference is dedicated to our colleagues who were onboard Malaysian Airlines Flight 17 on July 17th 2014. Their commitment and untiring efforts to eradicate HIV have advanced the scientific discovery for prevention, treatment, and cure.

Their important work shall continue on until the day that HIV is no more.

Pim de Kuijer, Lobbyist, Aids Fonds/STOP AIDS NOW!

Joep Lange, Co-Director of the HIV Netherlands Australia Research Collaboration (HIV-NAT)

Lucie van Mens, Director of Support at The Female Health Company

Martine de Schutter, Program Manager, Aids Fonds/STOP AIDS NOW!

Glenn Thomas, World Health Organisation

Jacqueline van Tongeren, Amsterdam Institute for Global Health and Development
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome</td>
<td>2</td>
</tr>
<tr>
<td>Scholarship Recipients</td>
<td>4</td>
</tr>
<tr>
<td>Scientific Organizing Committee</td>
<td>5</td>
</tr>
<tr>
<td>Agenda</td>
<td>6</td>
</tr>
<tr>
<td>Keynote Speaker</td>
<td>8</td>
</tr>
<tr>
<td>Plenary Speakers</td>
<td>10</td>
</tr>
<tr>
<td>Speakers (By Session)</td>
<td>14</td>
</tr>
<tr>
<td>Poster Presentation Abstracts</td>
<td>43</td>
</tr>
<tr>
<td>Host Organizations</td>
<td>60</td>
</tr>
<tr>
<td>Dinner and Reception</td>
<td>64</td>
</tr>
<tr>
<td>Special Events</td>
<td>66</td>
</tr>
<tr>
<td>Sponsors</td>
<td>68</td>
</tr>
<tr>
<td>Community Representation and Financial Support</td>
<td>72</td>
</tr>
</tbody>
</table>
WELCOME

On behalf of the Scientific Organizing Committee we would like to welcome you to our inaugural Conference on Cell and Gene Therapy for HIV Cure, a meeting designed to highlight and discuss the prospect of cell and gene therapy for the treatment and cure of HIV.

The very mission of the conference – to inspire collaboration, exploration and innovation – embodies the same virtues upon which Seattle was founded and continues to thrive. In 1851, Arthur A. Denny and his family left Cherry Grove, Illinois to travel westward in search of something better. Battling capricious weather and uncharted terrain, the Denny party along with other founding notables eventually built a settlement on mudflats between the mouth of the Duwamish River and Elliot Bay, which they named Duwamps. Hard work, determination, and lumber quickly transformed the town of Duwamps into the robust and thriving city of Seattle, renamed in honor of the great Duwamish Chief Sealth who became a close friend to many of the settlers.

Growth and prosperity are always tempered with setback - and so it was that in 1889 the gluepot of a local cabinet maker boiled over, ignited saw dust and turpentine, and quickly set fire to the entire city. The same resolve and courage that nurtured the creation of Seattle spurred the settlers to start rebuilding immediately. Undeterred by the ruin, the residents of Seattle saw the fire as an opportunity to make their beloved city bigger, better and stronger. Street by street, stone by stone.

It is this same hardy and undeterred spirit which continues to drive and define Seattle, inspiring the exploration of the unknown, reliance upon community and the fearless pushing of boundaries. It is embodied by the great icon of the 1962 World Fair, the Space Needle, designed and built to encourage people to think forward and dream of possibility; by Boeing and Microsoft, technological pioneers, who have allowed us to literally and figuratively expand our horizons into the clouds; in Starbucks and Amazon, who have dramatically influenced culture and added new dimensions to our daily lifestyles; and in visionary musicians like Quincy Jones, Jimi Hendrix and Nirvana, who have challenged convention and inspired generations.
And so like many before it, the Conference on Cell and Gene Therapy for HIV Cure finds its place amongst the explorers, collaborators and innovators of Seattle’s History. Like Seattle’s founders, we are inspired by possibility and motivated by resolve, as we work together to turn the hope of a cure into a tangible and viable intervention. Step by step, vision by vision. And, like our contemporaries, we hope that our shared work challenges convention and influences culture for the better so that one day the dream of an HIV free world will become a reality.

Keith R. Jerome, MD, PhD  
Co-PI, defeatHIV  

Hans-Peter Kiem, MD  
Co-PI, defeatHIV
SCHOLARSHIP RECIPIENTS

The Conference on Cell and Gene Therapy for HIV Cure is pleased to announce the 2014 scholarship recipients:

Laura Richert-Spuhler, PhD
George Llewellyn, PhD
Tiffany Hensley-McBain
Coline Exline, PhD
Biswajit Paul
Nixon Niyonzima, MD
Alison Dewey
Melanie Alvarado

Mayumi Takahashi, PhD
Maggie Bobbin
Sangeetha Satheesan, MSc
Debbie Ruelas, MSc
Mustafa Ghanem
Kamola Saydaminova, MSc
Maximillian Richter, MSc
Joumana Zeidan, PhD

Congratulations!
SCIENTIFIC ORGANIZING COMMITTEE

Paula Cannon, PhD  
Associate Professor, Molecular Microbiology & Immunology, Pediatrics, Biochemistry & Molecular Biology, Keck School of Medicine, University of Southern California  
defeatHIV Steering Committee

Philip Gregory, PhD  
Vice President, Research and CSO, Sangamo BioSciences  
defeatHIV Steering Committee

Keith R. Jerome, MD, PhD  
Host, Conference on CGT4HIVCure2014  
Co-PI, defeatHIV Martin Delaney Collaboratory  
Associate Member, Vaccine & 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 CGT4HIVCure2014  
Co-PI, defeatHIV Martin Delaney Collaboratory  
Associate Director, Program in Transplantation Biology  
Member, Clinical Research Division  
Affiliate Investigator, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center  
Professor of Medicine / Adjunct Professor of Pathology, University of Washington School of Medicine

Ronald Mitsuyasu, MD  
Director, Center for Clinical AIDS Research and Education at UCLA (CARE)  
Professor, Department of Medicine, Associate Director, UCLA AIDS Institute, University of California, Los Angeles  
defeatHIV Scientific Advisory Panel

Julie Overbaugh, PhD  
Member, Human Biology Division and Public Health Sciences Division, Fred Hutchinson Cancer Research Center  
Affiliate Professor, Department of Microbiology, University of Washington

Andrew Scharenberg, MD  
Professor, Pediatrics, Seattle Children’s  
Adjunct Professor, Immunology, University of Washington  
defeatHIV Steering Committee

Erick Seelbach  
HIV/AIDS Regional Resource Consultant, U.S. Department of Health and Human Services  
defeatHIV and CFAR Community Advisory Boards
### AGENDA | DAY 1: TUESDAY AUGUST 26, 2014

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00-8:00</td>
<td>Registration and Continental Breakfast</td>
</tr>
<tr>
<td>8:00-8:15</td>
<td>Conference Welcome: Keith R. Jerome, MD, PhD; Hans-Peter Kiem, MD</td>
</tr>
<tr>
<td>8:15-8:45</td>
<td>Plenary Speaker: Lawrence Corey, MD</td>
</tr>
<tr>
<td>8:45-9:15</td>
<td>SESSION 1 HIV Cure History and Challenges</td>
</tr>
<tr>
<td></td>
<td>Steven G. Deeks, MD</td>
</tr>
<tr>
<td>9:15-9:45</td>
<td>John A. Zaia, MD</td>
</tr>
<tr>
<td>9:45-10:15</td>
<td>M. Juliana McElrath, MD, PhD</td>
</tr>
<tr>
<td>10:15-10:30</td>
<td>Break</td>
</tr>
<tr>
<td>10:30-10:45</td>
<td>Keynote</td>
</tr>
<tr>
<td></td>
<td>Introduction: Keynote Speaker – Lawrence Corey, MD</td>
</tr>
<tr>
<td>10:45-11:45</td>
<td>Keynote Speaker: Françoise Barré-Sinoussi, PhD</td>
</tr>
<tr>
<td>11:45-1:00</td>
<td>Lunch: Weintraub B-Suites</td>
</tr>
<tr>
<td>1:00-1:30</td>
<td>SESSION 2 Vaccines and Antibodies</td>
</tr>
<tr>
<td></td>
<td>Galit Alter, PhD</td>
</tr>
<tr>
<td>1:30-1:45</td>
<td>Leonidas Stamatatos, PhD</td>
</tr>
<tr>
<td>1:45-2:00</td>
<td>Katherine Williams, PhD</td>
</tr>
<tr>
<td>2:00-2:30</td>
<td>SESSION 3 Inflammation</td>
</tr>
<tr>
<td></td>
<td>Nichole Klatt, PhD</td>
</tr>
<tr>
<td>2:30-2:45</td>
<td>Joumana Zeidan, PhD</td>
</tr>
<tr>
<td>2:45-3:00</td>
<td>Laura Richert-Spuhler, PhD</td>
</tr>
<tr>
<td>3:00-3:15</td>
<td>Break</td>
</tr>
<tr>
<td>3:15-3:45</td>
<td>SESSION 4 HIV Integration and Latency</td>
</tr>
<tr>
<td></td>
<td>Jonathan Karn, PhD</td>
</tr>
<tr>
<td>3:45-4:15</td>
<td>Jerome Zack, PhD</td>
</tr>
<tr>
<td>4:15-4:45</td>
<td>Lisa M. Frenkel, MD</td>
</tr>
<tr>
<td>4:45-5:00</td>
<td>Debbie Ruelas, MSc</td>
</tr>
<tr>
<td>5:00-5:15</td>
<td>Ann Chahroudi, MD, PhD</td>
</tr>
<tr>
<td>5:45-7:00</td>
<td>Posters, Cocktails and Hors d’Oeuvres</td>
</tr>
<tr>
<td>7:00-9:00</td>
<td>Dinner</td>
</tr>
<tr>
<td>9:00-11:00</td>
<td>Gallery Access</td>
</tr>
</tbody>
</table>
# DAY 2: WEDNESDAY AUGUST 27, 2014 | AGENDA

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:30-8:00</td>
<td>Registration and Continental Breakfast</td>
</tr>
<tr>
<td>8:00-8:30</td>
<td>Plenary Speaker: J. Keith Joung, MD, PhD</td>
</tr>
<tr>
<td>SESSION 5</td>
<td>Genome Editing</td>
</tr>
<tr>
<td>8:30-9:00</td>
<td>Paula Cannon, PhD</td>
</tr>
<tr>
<td>9:00-9:15</td>
<td>Colin Exline, PhD</td>
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<tr>
<td>9:15-9:30</td>
<td>George Llewellyn, PhD</td>
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<tr>
<td>9:30-9:45</td>
<td>Christopher W. Peterson, PhD</td>
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<tr>
<td>9:45-10:00</td>
<td>Michael C. Holmes, PhD</td>
</tr>
<tr>
<td>10:00-10:15</td>
<td>Break</td>
</tr>
<tr>
<td>SESSION 6</td>
<td>Vector Based Gene Therapy I</td>
</tr>
<tr>
<td>10:15-10:45</td>
<td>Ronald Mitsuyasu, MD</td>
</tr>
<tr>
<td>10:45-11:00</td>
<td>David L. DiGiusto, PhD</td>
</tr>
<tr>
<td>11:00-11:15</td>
<td>Bryan Burke, PhD</td>
</tr>
<tr>
<td>11:15-11:30</td>
<td>Scott G. Kitchen, PhD</td>
</tr>
<tr>
<td>11:30-1:00</td>
<td>Lunch: Weintraub B-Suites</td>
</tr>
<tr>
<td>SESSION 7</td>
<td>Vector Based Gene Therapy II</td>
</tr>
<tr>
<td>1:00-1:30</td>
<td>André Lieber, MD, PhD</td>
</tr>
<tr>
<td>1:30-1:45</td>
<td>Jennifer E. Adair, PhD</td>
</tr>
<tr>
<td>1:45-2:00</td>
<td>Edward Berger, PhD</td>
</tr>
<tr>
<td>2:00-2:15</td>
<td>Mustafa Ghanem</td>
</tr>
<tr>
<td>2:15-2:30</td>
<td>Fadila Bouamr, PhD</td>
</tr>
<tr>
<td>2:30-3:00</td>
<td>Hans-Peter Kiem, MD</td>
</tr>
<tr>
<td>3:00-3:15</td>
<td>CLOSING REMARKS</td>
</tr>
</tbody>
</table>
| Community Event | with Nobel Laureate Françoise Barré-Sinoussi, PhD   | In conversation with local journalist Rosette Royale, Dr. Françoise Barré-Sinoussi, PhD will discuss the current international efforts toward an HIV Cure and the global initiative, “Towards an HIV Cure” which addresses the need for engaging the communities of people living with HIV. Additionally, she will discuss how she has fought HIV by advocating for gay rights, by calling for the decriminalization of drug addiction, and by challenging the Vatican’s anti-condom views.

The FHCRC offers free wireless access via the FHCRC Guest Network.
Françoise Barré-Sinoussi, PhD
Professor, Institut Pasteur, Paris, France
Research Director at the INSERM, France
2008 Nobel Prize Laureate, Medicine

Prof. Françoise Barré-Sinoussi is the Director of the “Regulation of Retroviral Infections” Unit in the Department of Virology at the Institut Pasteur in Paris. She has been involved in retrovirology research since the early 1970’s and is widely recognized for her contributions to HIV/AIDS research, in particular in the discovery of HIV. The research programs of her team are focused on mechanisms required to protect against HIV/SIV infections and/or against early pathogenic signals induced by HIV/SIV.

Prof. Barré-Sinoussi has maintained a strong commitment to building international capacity, training and technology infrastructure with an emphasis on Africa and Asia. As such, she has been a leader in promoting integration between HIV/AIDS research and actions in
resource limited countries, in particular through the Institut Pasteur International Network and the coordination of the ANRS research programs in Cambodia and Vietnam. Prof. Barré-Sinoussi is author and co-author of more than 270 original publications. Throughout her career, she has received more than 10 national or international awards, including the Nobel Prize for Medicine in 2008 for her contributions to HIV/AIDS. In February 2009 she was elected member of the French Academy of Science, and has served as President of the International AIDS Society since July 2012.

On The Road Towards An HIV Cure

Prof. Françoise Barré-Sinoussi

Since the first cases of AIDS in 1981 and the identification of its etiological agent in 1983, much progress has been made in both the development of tools to prevent and treat HIV infection and the access to these tools. In particular, the wide array of antiretroviral treatments that now exists has considerably transformed the face of the infection from a lethal disease to a chronic condition. Today, thanks to unprecedented international efforts, people living with HIV have access to these life-saving treatments in resource-limited countries.

However, the sustainability of these life-long therapies is a real challenge both for the patients who have to meticulously take them each day and for the global economy considering their cost. Indeed, these antiretroviral treatments are not curative as HIV latently persists in reservoir cells and in many compartments of the host.

Novel therapeutic strategies that would cure HIV infection or at least induce a sustainable remission in patients without the need to further take medication are thus an absolute necessity. The increased knowledge of HIV reservoirs and of the mechanisms of persistence as well as reports of proof of concept studies, have generated a great optimism in the scientific community, which now believe that a sustainable remission of HIV infection is an achievable goal.

In 2010, the International AIDS Society has launched the “Towards an HIV Cure” initiative with the aim to mobilize the scientific community and accelerate research on this topic, which has since then become a priority in the HIV science agenda.
PLenary Speakers

Lawrence Corey, MD
President and Director Emeritus
Fred Hutchinson Cancer Research Center
Lawrence Corey Endowed Chair in Medical Virology
Professor, Laboratory Medicine and Allergy and Infectious Diseases, University of Washington

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

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

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

Corey received his medical degree from the University of Michigan and his infectious diseases training at the University of Washington. He has authored more than 600 scientific publications and has served on numerous editorial boards and national committees, along with serving as head of both the NIH-sponsored AIDS therapy and HIV vaccine programs.
Immunologic Approaches to HIV Cure

L. Corey
Fred Hutchinson Cancer Research Center, Seattle, WA

While the advent of combination therapy has been, perhaps, the most impressive therapeutic accomplishment in medicine of the last 20 years; the cost, difficulty with long term adherence, and the continued risk of the complications of chronic inflammation provide major impetus for the development of alternative methods of HIV control. The sexual mode of acquisition, the fact that the vast majority of acquisitions are asymptomatic, and the rapid establishment of latency means that prevention of the latent reservoir, even with early aggressive antiretroviral treatment, is unlikely. The Mississippi baby seems to corroborate this concept. As such, one needs an approach that will alter the long term behavior of latently infected T cells. Increasingly, there is evidence that many latently infected cells have HIV RNA detected and that some HIV proteins / peptides may be made and displayed on such cells. 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 can perform immune surveillance for cells that are transcribing HIV proteins or peptides on the infected cells. 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, and the targets can be to conserved regions of the virus. Thus, obviating selection of immune escape variants.

This talk will discuss the potential of such an approach for providing long term benefit for HIV control and the potential to allow long term treatment interruption for patients with chronic HIV.
J. Keith Joung, MD, PhD
Associate Chief of Pathology for Research,
The Jim and Ann Orr MGH Research Scholar
Director, Molecular Pathology Unit,
Massachusetts General Hospital
Associate Professor of Pathology,
Harvard Medical School

Dr. J. Keith Joung is a leading innovator in the field of genome editing. He currently serves as associate chief of pathology for research and the Jim and Ann Orr Research Scholar at Massachusetts General Hospital (MGH) and is an associate professor of pathology at Harvard Medical School. He also is director of the Molecular Pathology Unit and is a member of the Center for Cancer Research and Center for Computational and Integrative Biology at MGH. Dr. Joung has been a pioneer in the development of important technologies for targeted genome editing and epigenome editing of human cells. He has received numerous awards including an NIH Director’s Pioneer Award, an NIH Director’s Transformative Research Project R01 Award, the Jim and Ann Orr MGH Research Scholar Award, and election into the American Association of University Pathologists. He is a scientific co-founder of Editas Medicine, a company dedicated to the translation of genome editing technologies for therapy of human diseases.

Dr. Joung holds a Ph.D. in genetics from Harvard University, an M.D. from Harvard Medical School and an A.B. in biochemical sciences from Harvard College.
Targeted Genome and Epigenome Editing Using Engineered CRISPR-Cas and TALE Technologies

JK Joung
Massachusetts General Hospital & Harvard Medical School, Charlestown, MA

Targeted genome and epigenome editing technologies have recently emerged as important tools for biomedical research and as potential reagents for therapies of a broad range of diseases, including as a means for inducing resistance to HIV in human T-cells. In this talk, I will present our recent work on the clustered regularly interspaced short palindromic repeat (CRISPR) RNA-guided nuclease platform for introducing targeted genome sequence alterations, including discussion about the latest specificity improvements developed by our group. I will also briefly describe the creation and validation of new technologies for modifying specific epigenomic marks on histones and DNA that can be used to induce targeted alterations in endogenous human gene expression. Taken together, these methodologies provide transformative tools for understanding human biology and offer promising pathways forward for developing therapies based on targeted alterations of gene sequence and expression.
Steven G. Deeks, MD INVITED SPEAKER
Professor of Medicine, Division of HIV/AIDS, University of California, San Francisco

HIV Cure: Implications of Recent Advances and Setbacks
SG Deeks
University of California, San Francisco

There are several distinct pathways toward an HIV cure: (1) depleting the entire reservoir (e.g., “shock and kill”), (2) enabling long-term host-mediated control of HIV replication and (3) constructing an HIV-resistant immune system using via cell- and gene-therapies. Recent heroic interventions such as hematopoietic stem cell transplant and very early initiation of antiretroviral therapy suggest that dramatic reductions in the reservoir size can be achieved, but that complete eradication will be difficult if not impossible to achieve. Most attempts to stimulate effective host-mediated control of HIV have failed. The study of elite and post-treatment controllers suggest that long-term disease remission might be possible, but that such a state will require both a low reservoir size and a durable means by which the host can control the persistent population of replication-competent HIV. The implications for recent advances and setbacks in achieving HIV remission will be discussed.
Hematopoietic Stem Cell Therapy for HIV/AIDS: the Dream and the Possibility

JA Zaia¹ and D DiGiusto¹
¹Department of Virology, Beckman Research Institute of City of Hope, Duarte CA.

Background: The possibility of creating a disease-resistant immune system by transplantation of autologous, HIV-1-resistant hematopoietic stem and progenitor cells (HSPC) has been nurtured by select case studies in man and animal models of disease. HSPC transplantation (HCT) of either naturally HIV-resistant stem cells (CCR5\(\Delta^{32/\Delta^{32}}\)) or genetically modified (GM)-HSPC have both been shown to control and even cure HIV-1 infection. The concept of attacking the problem of minimum residual disease (MRD), i.e. the HIV-1 reservoir, with HCT resonates with oncologic MRD strategies. At present, however, this approach is useful only as a test of concept and is not thought to be readily applicable to treatment of most HIV/AIDS patients. For GM-HSPC therapy to become a viable approach to HIV-1 management or cure, new methods must be developed that are safe, scalable, effective in controlling HIV-1 (in the absence of antiretroviral therapy) and affordable. Most importantly, therapy must be amenable to administration through local health centers without the need for complex manufacturing facilities or procedures that require advanced levels of pre- and post-infusion care.

Subject Matter: The results from several pilot clinical studies in HIV-1 patients and the current strategies to develop GM-HSPC for HIV-1+ patients are reviewed. Important issues related to methods of genetic modification, stem cell source, patient selection, pre-infusion conditioning and post-infusion cell selection are discussed.

Conclusions: Critically important trials are now in progress or in advanced planning stages, and if successful, these 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 GM-HSPC technology into a readily applicable strategy for control of HIV-1 infection. A vision of how the field must progress to yield a viable GM-HSPC therapy for cure of HIV-1 is provided.
Contributions of Vaccines and Immune Modulation in HIV-1 Clearance

M. Juliana McElrath, MD, PhD
Sr. Vice President, Director & Member, Vaccine & Infectious Disease Division, Fred Hutchinson Cancer Research Center
Professor, Department of Medicine, Global Health & Laboratory Medicine, University of Washington

HIV-1 forms stable, latent reservoirs in resting memory CD4+ T cells that prevent clearance of infection. This occurs despite the use of highly potent combination antiretroviral therapy during acute infection. Thus far, HIV-1 vaccine regimens evaluated post-infection may induce enhanced immune responses but have not been successful in persistent control of viremia or HIV-1 provirus levels. Recent preclinical studies with persistent recombinant CMV vectors and passive immunoprophylaxis provided before or during acute infection provide compelling data that potent effector responses armed at the time of exposure and throughout infection may have some role in reducing the reservoir. These findings will be highlighted, along with other immunomodulation strategies that may hold promise in purging the HIV-1 reservoir in humans.
Eradication of HIV-1 Reservoirs with Antibody Mediated Killing

Z Euler and G Alter

The Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University

**Background:** Up to date there is no effective cure for HIV-1. The ‘shock and kill’ approach, aims to reactivate the reservoir and target its clearance via immune or therapeutic mechanisms. Beyond T cells, purging the reservoir after reactivation with HIV-specific antibodies that recruit the cytotoxic and antiviral activity of the innate immune system has not been assessed. It has been shown that functional antibodies have a protective effect in HIV-disease progression and were induced in the RV144 vaccine trial. Finding epitopes targeted by functional antibodies on reactivated latent cells as well as the specific functions that lead to best killing of the reservoir could lead to the design of new therapeutic approaches aimed to eradicate HIV-reservoirs.

**Methodology:** Latently infected cell lines (ACH2, J89GFP and U1) and primary PBMCs from HAART treated patients, were treated for 24 hours with PMA, CD3/CD28 or histone deacetylase inhibitors (HDACi); Vorinostat, Panobinostat and Romidepsin and incubated with various monoclonal antibodies (mAbs) to Env (n=30) for the detection of various epitopes on reactivated cells. Intracellular p24 or GFP and extracellular Env were detected with flow cytometry. An Antibody Dependent Cellular Phagocytosis (ADCP), an antibody cellular cytotoxicity (ADCC), and antibody complement activation (ADCAA) assay was performed on reactivated cells. Killing of latently infected cells in primary PBMCs was quantified by provirus DNA quantification.

**Results:** Latency reversal in all cell types led to an upregulation of surface ENV, including on primary treated memory CD4+ T cells. More critically, a clear hierarchy was observed among tested mAbs in their ability to recognize reactivated cells, with 2G12 recognizing the largest fraction of reactivated cells in the cell lines. However marked differences emerged in the capacity of neutralizing versus non-neutralizing antibodies to recognize reactivated target cells. Moreover, distinct hierarchies emerged in the capacity of distinct antibodies in driving ADCP, ADCC, and ADCAA, and latent reservoir killing.

**Conclusions:** Here we show that HDACi reactivated latently infected cells robustly expressed Env on the cell surface. More critically, a clear hierarchy was observed among tested mAbs in their ability to recognize reactivated cells, and recruit innate effector activities that can promote efficient killing or eradication of the reservoir. These findings provide a critical footprint for epitopes that a therapeutic vaccine or passive transfer antibodies needs to target as well as potential functional epitopes that may relevant for driving rapid elimination of the reservoir, providing a path to a functional cure.
## Immunogen-Design Efforts to Activate the Germline BCR Forms of Broadly Neutralizing Antibodies Against HIV-1

**AT McGuire¹, AM Dreyer¹, S Carbonetti¹, A Lippy¹, J Glenn¹, JF Scheid², H Mouquet³, and L Stamatatos¹**

¹Seattle Biomedical Research Institute, Seattle, WA; ²Laboratory of Molecular Immunology, The Rockefeller University; New York, NY; ³Laboratory of Humoral Response to Pathogens, Department of Immunology, Institut Pasteur and CNS-URA 1961, Paris, France

**Background:** Anti-HIV-1 Env broadly neutralizing antibodies (bNAbs) have been isolated from infected subjects; they protect animals from experimental infection and are expected to be a key component of an effective vaccine. During Env-immunization, however, antibody responses with narrow neutralizing breadth (nNAbs) are generated, despite the fact that they target Env regions also targeted by bNAbs.

**Methods:** To understand the underlying mechanisms for the preferential elicitation of nNAbs during Env-immunization, we screened a panel of recombinant Env proteins for binding to germline-reverted antibodies and discovered that in contrast to bNAbs, nNAbs targeting both the CD4-BS and the V3 recognized diverse recombinant HIV-1 Envs. In addition, we investigated the competition for Env between B cells expressing germline BCRs of bNAbs and B cells expressing germline BCRs of nNAbs, targeting overlapping epitopes on an Env that is capable of binding to, and activating B cell expressing glVRC01 class BCRs.

**Results and Conclusion:** We observed that due to differences in Env-binding affinities, nNAb B cell progenitors have an inherent advantage over bNAb progenitors in becoming activated and internalizing Env. Importantly, for HIV-1 vaccine development efforts we identified an immunogen-design strategy to minimize the activation of BCRs of nNAbs that bind overlapping epitopes to those of bNAbs.
Katherine Williams, PhD  
*Research Associate, Human Biology Division, Fred Hutchinson Cancer Research Center*

**B Cells That Bind HIV Particles Encode CD4-Induced, C11-Like and V3-Specific Antibodies That Mediate Broad ADCC Activity**

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**Background:** HIV-specific antibodies (Abs) reduce viral burden by preventing infection of CD4+ T cells or tagging virally infected cells for destruction through a mechanism known as antibody-dependent cellular cytotoxicity (ADCC). ADCC antibodies are present before neutralizing antibodies and thus may play a role in early infection. Evidence from in vivo observations, including macaque studies, natural infection cohorts and vaccination studies in humans have identified a relationship between plasma-mediated ADCC activity, reduced viremia levels and decreased transmission. Characteristics associated with broadly neutralizing antibodies (bnAbs) have been well-defined, however far less is known about the in vitro characteristics that define powerful ADCC-mediating antibodies.

**Methods:** We employed fluorescently-labeled viral like particles (VLPs) generated with a combination of two clade A envs to sort IgG+ memory B cells obtained 2.5 years following HIV infection in QA255, a patient who developed a broad neutralizing and ADCC-mediating antibody response early following infection.

**Results:** In total, 48 Abs were produced from 192 sorted B cells that bound VLPs. Thirteen Abs demonstrated measurable binding to Q461.d1 VLP by ELISA. Three Abs neutralized Tier 1 viruses, though none neutralized Tier 2 viruses. Analysis using the rapid and fluorometric ADCC assay determined that three clonally unrelated Abs mediated potent ADCC activity against both of the Clade A envs used in the VLP sort as well as envs from additional A, B and C clades. Two of the three Abs recapitulated the majority of the heterologous, cross-clade ADCC breadth observed with contemporaneous QA255 plasma. Experiments using Fabs to block epitope-specific ADCC activity indicated that none of the three antibodies resemble A32, an antibody that is typically a dominant ADCC response in naturally infected individuals. Rather, similar experiments and gp120-based ELISA data suggest that two of the Abs identified target a more obscure, C11-like epitope and the third targets a V3-specific linear epitope.

**Conclusions:** Here we identify two clonally unrelated ADCC-mediating antibodies following a B-cell sort with VLPs that target a similar epitope and demonstrate comparable breadth but different levels of ADCC-mediating potency. Because ADCC-mediating antibodies can eliminate HIV-infected cells, these antibodies could augment other approaches to reduce the viral reservoir.
Inflammation, microbial translocation, and mucosal dysfunction in HIV persistence

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With more than thirty million HIV-infected individuals worldwide, containment and eventual eradication of the AIDS pandemic remains a top priority in contemporary biomedical research. While antiretroviral therapy (ART) can improve health during HIV infection, a cure is not yet available, and despite suppression of viremia with ART, these individuals still have increased morbidity and mortality compared to uninfected individuals. Indeed, HIV-infected subjects cannot discontinue ART, because residual HIV persists, and virus rebound is inevitable if ART is terminated. The HIV reservoir is complex, and is closely associated with, and potentially driven by, immune activation and inflammation during HIV infection. Indeed, HIV infection results in a vicious cycle of mucosal damage, chronic inflammation and overall immunological dysfunction, which are closely associated with disease. This chronic immune activation is also strongly associated with gastrointestinal (GI) mucosal damage and microbial translocation, which do not resolve completely with ART. While there is a clear positive correlation between measures of immune activation and HIV persistence in ART-suppressed individuals, the role of immune activation and mucosal dysfunction as either the cause and/or consequence of HIV persistence is unknown.
The Inflammatory Environment Before and After Adoptive Transfer of ZFN CCR5 Modified CD4 T-cells (SB-728-T) in HIV Subjects Impacts Reconstitution of T Memory Stem Cells and Central Memory Cells

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The inflammatory environment before and after adoptive transfer of ZFN CCR5 modified CD4 T-cells (SB-728-T) in HIV subjects impacts reconstitution of T memory stem cells and central memory cells.

Background: 9 aviremic HIV+ subjects on ART with CD4 counts between 200-500 cells/mm3 received 10-30 billion SB-728-T cells. Sustained CD4 increases in peripheral blood were observed in all treated subjects (median +103 cells/mm3 at 1 yr). CCR5-modified cells expanded and persisted (median = 2.1% in PB 3 yrs post infusion). Long-term persistence of CCR5-modified cells suggested their presence within long lived CD4 populations, such as central memory T-cells (TCM) and T memory stem cells (TSCM).

Methods: TSCM phenotyping (CD95, CD58) was performed on PBMCs pre- and post-infusion (up to 3 yrs). CCR5 modification level within CD4 subsets was determined by qPCR. Markers associated with T-cell activation (LAG-3/PD-1, Ki67) were assessed on CD4 and CD8 subsets, and innate cell activation (HLA-DR/CD40/CD86, and PD-L1) were assessed on monocyte subsets.

Results: We had previously shown that TCM increased significantly post-infusion and correlated with improved CD4 counts (r=0.87, p=0.0045). We have now identified a novel TSCM CD4 subset characterized by co-expression of low levels of CD45RA and CD45RO, expression of CD27, CCR7 and the memory stem cell markers CD95 and CD58. These cells were highly enriched in CCR5 modification (23.2% ± 17.6) compared to TCM (2.4% ± 1.84) at 3 yrs and positively correlated with CD4 reconstitution (r=0.7904, p=0.0279). Baseline levels of monocyte activation (HLA-DRhiCD86hiCD40+) inversely correlated with TSCM counts (r=-0.7882, p=0.0116) and levels of CD4 T-cell reconstitution (r=-0.8992, p=0.002) at 3 yrs post-infusion. Gene analysis of total innate cells (CD3-CD19+HLA-DR+) showed differential expression of Interferon Stimulated Genes (ISGs) in subjects with higher immune reconstitution. Post-infusion levels of PD-1 and PD-L1, two ISGs, on CM and classical CD14++CD16- monocytes inversely correlated with long-term levels of CM reconstitution (r=-0.8833, p=0.0031).

Conclusions: Generation of long-lived TSCM by SB-728-T is a potential mechanism by which CCR5-modified cells can persist for years post-infusion. Our results highlight the negative impact that pro-inflammatory cells and pathways exert on engraftment of TSCM and long-term reconstitution of the critical TCM compartment, a surrogate marker of slow disease progression. These results should provide guidance for identifying HIV-infected individuals who may have a more intact immune system and thus more suitable for HIV immunotherapy.
Rapid Loss of TH17 Cells After Acute SIV Infection May Underlie Mucosal Dysfunction

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Background: During chronic HIV/SIV infection, CD4+ IL-17-producing T cells (TH17) are significantly depleted from mucosal tissues and their absence is highly associated with gastrointestinal (GI) dysfunction. However, the kinetics of immune dysfunction, the relationship of such dysfunction to microbial translocation, and how this dysfunction may affect the establishment of the viral reservoir remain unknown. Herein, we elucidate the kinetics of acute host/pathogen interactions with the goal of identifying early events after HIV infection that could reveal potential targets of therapeutic strategies.

Methods: We collected longitudinal biopsies and blood at early acute timepoints from six rhesus macaques following intrarectal SIV challenge with SIVmac239x (100,000 TCID50). Rectum, colon, jejunum, lymph nodes, and blood were collected pre-SIV and days 3, 7, 14, 28, 42, and 63 post-challenge. Parameters reflective of immunophenotype, function, inflammation, viral kinetics, and microbial translocation were measured.

Results: Strikingly, TH17 cells were significantly depleted from all GI tract sites by day 3 post-SIV infection (rectum: p=.005; colon: p=.032; jejunum: p=.047), but such depletion was not associated with generalized CD4 depletion, viral load, or generalized loss of cytokine production (e.g., related to exhaustion/anergy). The marked loss of TH17 cells appeared to be unique to this particular subset, as we did not observe early loss of IL-17 production in other immune cells. Additionally, we found that loss of TH17 cells preceded overt inflammation and microbial translocation, indicating that this deficiency may underlie the subsequent mucosal dysfunction observed during HIV/SIV.

Conclusions: Taken together, our data indicate that the loss of TH17 cells from the GI tract at the earliest stages of infection uniquely precedes microbial translocation and a systemic proinflammatory state. Immediately targeting the preservation and/or restoration of TH17 cells may accordingly serve as a novel therapeutic approach to combat chronic HIV infection, the establishment of the viral reservoir, and downstream pathologies.
Signaling Pathways and Epigenetic Mechanisms Controlling HIV Latency

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HAART must be continuously maintained prevent HIV-1 rebound from latent reservoirs. Factors that are responsible for maintenance of HIV-1 latency were identified by independent genome wide shRNA library screens. The mechanism of action of compounds that reactivate HIV proviruses was studied by comparison to T-cell receptor activation pathways. shRNA library screens were performed in Jurkat T-cells. Cells carrying reactivated proviruses were purified by sorting after sequential passages and the shRNA sequences identified by next-generation sequencing and classified by systems biology tools. The screen identified many of the shRNAs associated with epigenetic silencing mechanisms, as well as some novel targets including the estrogen receptor, ESR1. In cells where PRC2 (EZH2) was knocked down, all of the inducible proviruses were found in the H3K27me3 population suggesting that PRC2 complex is required not only for maintenance of latency but also for entry of HIV proviruses into latency. The screening hits are being verified in primary cell models of HIV latency. Naïve T cells were polarized into Th17, Th1, Th2 and Treg cells, infected with a single round HIV-1 proviral clone and forced into quiescence by cytokine restriction to generate latently infected cell populations. High content assays using imaging flow cytometry have shown the need to activate both P-TEFb and reverse epigenetic blocks. Evaluation of the mechanisms of action of the T-cell receptor, cytokines, disulfiram, farnesyl transferase and ESR1 inhibitors highlights how activation of unique pathways can lead to proviral reactivation. In conclusion, the reactivation of latent proviruses requires both P-TEFb and transcription initiation. Reversing any number of rate limiting steps can lead to proviral reactivation but in a limited number of cells. This suggests that there will be many opportunities to identify synergies between different classes of proviral activators and design efficient reactivation strategies.
SESSION 4 SPEAKERS

Jerome Zack, PhD **INVITED SPEAKER**

*Professor, Departments of Medicine, Microbiology, Immunology & Molecular Genetics, Associate Director, UCLA AIDS Institute, University of California, Los Angeles*

*A Strategy To Purge Latent HIV Reservoirs*

JA Zack, PA Wender, LH Rome, E Lowe, MD Marsden

¹David Geffen School of Medicine at UCLA; ²Stanford University

**Background:** Current antiretroviral therapy (ART), while highly effective against HIV, is not curative. Stable residual reservoirs of virus remain for years even in individuals with undetectable viremia. The best characterized and largest of these is the latent reservoir in resting memory CD4 T cells, which can re-kindle active virus replication following cessation of ART. To cure HIV disease, this reservoir must be eliminated.

**Methods:** Our group has been optimizing an “activation/elimination” strategy whereby latent virus expression is activated by modest stimulation of the host cell. Therapeutic agents are then administered, which directly kill those cells that have been newly induced to express virus, allowing more efficient depletion of the latent reservoir.

**Results:** We have designed and synthesized improved activating agents that target protein kinase C (PKC), and incorporated PKC modulators, along with anti-retroviral drugs (to provide an extra measure of safety), into novel natural nanoparticles that we will target specifically to CD4+ cells. In this way off-target activation of cell subsets that would not harbor latent virus can be avoided, which should reduce potential toxicity due to over-activation of immune cells. Furthermore we have developed a means of genetically enhancing CD8 T cells to kill latently-infected cells newly induced to produce virus.

**Conclusions:** We intend to combine these approaches and assess their efficacy in humanized mice, with the eventual goal of eradicating HIV from the body. If successful these studies may contribute to a strategy that proves useful in the treatment of HIV infection.
Lisa M. Frenkel, MD **INVITED SPEAKER**  

Professor, Pediatrics and Laboratory Medicine, University of Washington  

Co-Director, Center for Global Infectious Disease Research, Seattle Children’s Research Institute

**Persistence of HIV During Suppressive ART Linked to Integration into Specific Genes and to Low-Level HIV Replication**

L.M. Frenkel  
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**Background:** Combination antiretroviral therapies (ART) can suppress HIV RNA to undetectable levels in the blood. Yet if ART is stopped the virus typically resumes replication to pretherapy levels. Insights into the mechanisms that sustain the latent HIV reservoir during effective ART are needed to facilitate development of interventions to eliminate or contain the virus in the absence of ART.

**Methods:** To better understand events that allow HIV to persist despite ART, viral populations and cellular integration sites were studied in multiple individuals over 2-15 years of suppressive ART. To evaluate the selective pressures imposed by the immune system and/or antiretroviral drugs, the HIV population in the blood and some individuals’ genital tract were characterized by single genome amplification and sequencing of HIV envelop and polymerase genes. Viral integration sites were defined using a novel method that sequenced from HIV env through the 3' integration site, which also detected 1- and 2-long-terminal-repeat (LTR) circular forms generated from aberrant cycles of replication.

**Results:** Across several cohorts with HIV replication suppressed below the limits of plasma HIV RNA quantification by clinical assays, a spectrum of viral replication was evident by a variety of criteria. The subtlest evidence was increasing numbers of LTR forms, which were detected in all subjects evaluated, including those without evidence of viral sequence evolution. Studies of integration sites showed that proliferation of infected-cells contribute to the persistence of HIV, and appears to contribute to the low-level production of virus in the plasma and genital tract. During suppressive ART, HIV integration sites were overrepresented in genes associated with cancer and regulation of cell proliferation, and a greater proportion of persisting proviruses were in proliferating cells. Furthermore, persistence of cells with HIV integrated into certain genes was found across multiple participants.

**Conclusions:** Evidence of HIV replication was detected across all subjects, and HIV integration into specific genes appears to disrupt cell regulation in a manner that allows proliferation of infected cells. As cure strategies are designed, the promulgation of the HIV reservoir during suppressive ART by both continued HIV replication and the proliferation of infected cells should be considered.
Debbie Ruelas, MSc
Graduate Student, Gladstone Institute of Virology and Immunology, University of California, San Francisco

MicroRNA-155 Reinforces HIV Latency by Downregulating the TRIM32 Viral Activator

DS Ruelas, JK Chan, E Oh, AJ Heidersbach, AM Hebbeler, L Chavez, E Verdin, M Rape, WC Greene

Background: Achieving a cure for HIV will require both the complete suppression of active viral replication and the clearance of the transcriptionally silent proviral latent reservoir. Current drug treatments effectively target the active virus but leave the latent reservoir intact. We describe a cellular pathway involving miR-155 and one of its many cellular targets, TRIM32, that appears to promote a return to latency in reservoir cells transiently producing virus.

Methods: We first assessed whether miRNAs play a role in maintenance of viral latency by separately knocking down the expression of two of the major enzymes involved in miRNA biogenesis, DGCR8 and Dicer, in J-Lat 5A8 cells. We next used miRNA TLDA analysis to identify specific miRNAs that alter the level of reactivation following stimulation. We focused on miR-155 because its reintroduction into Dicer-deficient cells was able to rescue the level of latent reactivation in J-Lat 5A8 cells to the greatest extent, suggesting that it plays a prominent role in reinforcing HIV latency. We confirmed TRIM32 as a novel target of miR-155 using luciferase binding assays. An IκB kinase (IKK) kinase assay revealed that TRIM32 acts downstream of the IKKs. Finally, our in vitro ubiquitination assays demonstrate that TRIM32 is able to directly ubiquitinate IκBα.

Results: MiR-155, which is expressed at high levels in activated cells, impairs the expression of TRIM32, which normally serves as an HIV-activating agent. TRIM32 activates latent HIV by stimulating nuclear translocation of NF-κB. However, our studies reveal that TRIM32 activates NF-κB in a novel manner involving direct ubiquitination of IκBα. Specifically, TRIM32 induction of NF-κB proceeds independently of IKK activation within signalosomes.

Conclusion: Our studies of the potential role of microRNAs in the regulation of HIV latency have led to the identification of miR-155 and its inhibition of TRIM32 activation of NF-κB as events that promote the reestablishment of HIV latency in reservoir cells undergoing transient viral production.
**Background:** Despite many advances in AIDS research, a cure for HIV infection remains elusive. A key obstacle to the development of a cure for HIV is an incomplete understanding of the cellular and anatomic nature of viral reservoirs in the setting of successful ART. To address this gap in understanding, we developed a model of autologous hematopoietic stem cell transplantation (autoHSCT) in SHIV-infected, ART-treated rhesus macaques (RM) to interrogate the origin of the SIV reservoir.

**Methods:** This study included three SHIV-infected RM who received autoHSCT and three SHIV-infected control RM. The transplant procedure involved pre-infection G-CSF mobilization of CD34+ HSC followed by leukapheresis and cryopreservation of collected cells. All RM were then infected i.v. with RT-SHIV. Four weeks post-infection a potent ART regimen including PMPA, FTC, Efavirenz and Raltegravir was initiated. The experimental RM received total body irradiation (TBI) followed by infusion of HSC collected pre-infection. ART was interrupted 40 to 80 days post-transplant.

**Results:** As expected, the ART regimen reduced plasma SHIV-RNA levels to below 100 copies/ml in all six SHIV-infected RM. TBI was myeloablative as measured by circulating platelet, white blood cell, and lymphocyte counts and, following autoHSCT, engraftment was successful in all three transplanted RM. A rapid viral rebound was observed in plasma in the non-transplanted control RM as early as one week post-ART interruption. Despite TBI and transplant with pre-infection HSC, plasma SHIV-RNA rebound was also observed post-ART interruption in two out of three transplanted RM. In the third transplanted animal, while plasma SHIV-RNA and SHIV-DNA in bulk PBMCs remained undetectable at week two post-ART interruption, SHIV-DNA could be detected in sorted circulating CD4+ T-cells, spleen and lymph nodes.

**Conclusion:** To the best of our knowledge, this model of SHIV/autoHSCT has not been attempted before. This experiment demonstrated that TBI with autoHSCT is a feasible intervention that can lead to a reduction of the virus reservoir in the peripheral blood, but was not sufficient to induce a functional cure under these experimental conditions. Further studies using this experimental in vivo platform should be performed to test innovative interventions aimed at curing HIV infection in humans.
SESSION 5 SPEAKERS

Paula Cannon, PhD INVITED SPEAKER
Associate Professor, Molecular Microbiology & Immunology, Pediatrics, Biochemistry & Molecular Biology, Keck School of Medicine, University of Southern California

Giving HIV the [Zinc] Finger: Targeted Nucleases as Anti-HIV-Therapies

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Targeted nucleases, which include zinc finger nucleases (ZFNs), TALENs, homing endonucleases and the CRISPR/Cas9 system, offer the potential for site-specific gene engineering. By taking advantage of host cell repair pathways, the reagents can be used to achieve targeted gene knockout, gene editing, or gene addition. For anti-HIV applications, nucleases are being developed to disrupt the CCR5 or CXCR4 co-receptor genes, to promote the site-specific addition of anti-HIV genes at a designated site, or to target the HIV genome itself in order to disrupt the integrated provirus in infected cells. CCR5 gene knockout by ZFNs is the most clinically advanced application which is currently being used to engineer autologous T cells from HIV-infected individuals. Ongoing work in our group is directed towards a clinical trial based on hematopoietic stem cell (HSC) engineering with the same CCR5 ZFNs. Beyond this application we are using nucleases to improve the potential for site-specific gene editing in HSC, as well as exploring ways to deliver the reagents to relevant target cells in vivo. We use various humanized mouse models to help us towards these goals. Work-in-progress and current challenges will be presented.
Colin Exline, PhD
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Efficient Site-Specific Gene Editing in Hematopoietic Stem Cells as a Potential Gene Therapy for HIV

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Zinc finger nucleases (ZFN) can disrupt the CCR5 co-receptor gene in human HSC, giving rise to progeny CD4+ T cells resistant to CCR5-tropic HIV. However, CCR5-disrupted cells remain susceptible to CXCR4-tropic HIV, as do cells where only a single CCR5 allele is disrupted. We are therefore interested in developing methods to introduce additional genes at a disrupted CCR5 locus to further inhibit HIV.

ZFNs create double strand DNA breaks at specific sites that are repaired using either the cellular NHEJ or homology directed repair (HDR) pathways. By providing a DNA donor template homologous to the region surrounding the DNA break site, the HDR pathway can be co-opted specifically integrating additional genes at the disrupted locus during repair. The efficiency of such site-specific integrations can be monitored by including a GFP reporter in the donor. However, since introduction of foreign DNA into cells is sensed, triggering innate anti-viral pathways, the efficiency of this process can be limited by cytotoxicity.

Using mRNA electroporation to deliver ZFNs to HSC, coupled with transient incubation of cells at 30°C, we achieve CCR5 disruption rates that are >50%. DNA donor sequences can be introduced as either plasmid DNA, minicircle DNA or rAAV vector. Effects on cell viability are assayed by in vitro assays for acute cytotoxicity, or more extensive in vivo assays using engraftment of humanized mice. Additionally, immediate effects on interferon expression, downstream ISGs and other components of the intrinsic immune response are measured. These analyses identified minicircle formats as being significantly less toxic to HSC than analogous plasmid donors, leading to higher rates of site-specific insertion. Finally, rAAV vectors based on serotype AAV6 proved highly effective at transducing HSC and promoting gene addition, leading to levels of 20% stable gene expression when combined with ZFN mRNA, with minimal toxicity. These high levels of gene addition were maintained in progeny cells that grew out in humanized mice, suggesting that rAAV6 donors provide an effective platform to deliver homology templates to human HSC, and opening up the possibility of inserting additional anti-HIV genes at the CCR5 locus to protect against a more extensive panel of viruses.
Next generation TALENs mediate efficient disruption of the CCR5 gene in human HSCs and reduce off-target effects

The failure of antiretrovirals to completely clear HIV infections, combined with their long-term toxicities, highlight the need for more sophisticated therapeutic approaches that can enable permanent cures. Seeking to replicate the cure of the ‘Berlin Patient’ following hematopoietic stem cell (HSC) transplantation from a CCR5delta32 donor, we are developing strategies for efficient CCR5 disruption in human HSCs using TALE-nucleases (TALENs). These proteins are a class of engineered endonucleases containing a DNA recognition domain from bacterial TALE proteins linked to the endonuclease domain of the FokI restriction enzyme. Two appropriately matched TALENs will dimerize at a target site and create a double-stranded break that leads to gene disruption via error-prone non-homologous end joining. The TALE DNA recognition domain contains a central core of 33-35 amino acid repeats that each recognize a single base of DNA through a repeat variable di-residue (RVD) motif at positions 12 and 13. The four RVDs that occur most commonly in natural TALEs (NI, HD, NN and NG) provide a simple code that allows sequence-specific targeting modules to be constructed.

We tested TALEN constructs targeted against two different sites in CCR5: an N-terminal site, previously shown to support potent anti-HIV activity in vivo (site 160), and a site approximating the location of naturally occurring CCR5delta32 mutation (site 546). TALEN pairs targeted to site 546 were found to be the most active, and in particular those that used a C-terminal truncation considerably shorter (17 residues) than that described in most TALE studies (63 residues). Gene modification levels using the shorter truncation were significantly higher than achieved with the longer one, enabling modification of up to 65% of CCR5 alleles in primary human HSCs. For applications beyond research, TALENs using this more potent architecture must also provide exquisite specificity genome wide. As a first step toward generating TALEN proteins with improved specificity, we also tested the ability of alternative non-canonical RVDs to support TALEN activity. Using a panel of previously characterized and novel RVDs with pre-defined base preferences, we found that it was possible to completely replace the natural code RVDs with non-canonical ones and retain comparable on-target activity. Importantly, the alternative code RVDs displayed a reduction in off-target cleavage. Together these data provide a framework for the development of improved TALENs for genome editing applications in HSC.
Christopher W. Peterson, PhD
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Gene Editing of CCR5 in Hematopoietic Stem Cells in a Nonhuman Primate Model of HIV/AIDS

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Background: Hematopoietic stem cell (HSC) transplantation remains the only clinically observed path to functional cure of HIV infection. To better understand the mechanism of HSC-driven HIV control, and apply this therapy to a greater number of patients, we have developed a model of cART-suppressed HIV infection in the pigtailed macaque, applicable to both gene therapy- and allogeneic transplant-based cure strategies. Following transplantation of HIV-resistant, autologous cells into conditioned animals, we are evaluating the extent to which protected cell progeny impede infection by SIV/HIV (SHIV) chimeric virus in vivo.

Methods: Animals are challenged with SHIV virus containing an HIV envelope, after which a 3-drug cART regimen is initiated. Autologous HSCs are engineered to resist infection through targeted disruption of the CCR5 genetic locus using Zinc Finger Nucleases (ZFNs). Engraftment, persistence, and SHIV response of these autologous stem cells, and stem cell-derived lymphoid and myeloid cells, are measured in vivo.

Results: SHIV infection in the pigtailed macaque model results in sustained vireemia with consequent reduction in CD4+ T cells. Moreover, administration of three-drug cART leads to rapid and durable suppression of plasma viremia to <30 copies/mL plasma - suggesting that this model recapitulates key features of HIV infection and treatment in humans. CCR5 targeting experiments yield up to 60% gene disruption in CD34+ cells ex vivo, translating to approximately 5% disruption in vivo following transplant. Importantly, up to 10% of transplanted cells carry two disrupted alleles of CCR5; these cells should preferentially reconstitute CD4+ T-cell pools and other susceptible subsets following SHIV challenge. Consistent with this prediction, our preliminary data suggest that CCR5-deleted cells undergo positive selection following SHIV challenge in vivo.

Conclusions: Our pigtailed macaque model of HIV infection and cART represents a promising platform for modeling functional cure strategies. Here we show that CCR5 deletion does not impair HSC engraftment or differentiation, and that CCR5-deleted cells can undergo SHIV-dependent positive selection even when present at low levels. Our model enables the evaluation of novel therapeutic approaches in the clinically relevant context of cART controlled SHIV infection - a setting of particular importance to approaches aimed at addressing the viral reservoir.
Michael C. Holmes, PhD  INVITED SPEAKER
Senior Director, Therapeutic Gene Modification, Sangamo BioSciences, Inc.

Genome Editing with Zinc Finger Nucleases

MC Holmes
Sangamo BioSciences Inc, Richmond CA

The ability to engineer precise genetic modification of human stem cells would both accelerate research and extend the range of their potential therapeutic application. This possibility is now being realized via the use of zinc finger nucleases (ZFNs). ZFNs are customizable, sequence-specific endonucleases that can be designed to introduce a discrete cleavage event at any user-chosen location within the stem cell genome. We have previously shown that ZFNs targeting CCR5, an obligate co-receptor for entry of R5-tropic HIV, render modified CD4 T cells resistant to HIV analogous to cells from subjects homozygous for a naturally occurring CCR5 mutation (CCR5Δ32). This talk will describe the recent clinical results with ZFN-modified CD4 T cells as well as preclinical proof-of-concept studies towards the development of autologous, CCR5-disrupted CD34 stem cells as potential treatments for HIV.
Gene therapy for individuals infected with HIV has the potential to provide a once-only or limited treatment that will act to reduce viral load, preserve the immune system, and mitigate cumulative toxicities associated with highly active antiretroviral therapy (HAART). Several prototypic trials have evaluated the possibility of utilizing peripheral T-cells/monocytes and hematopoietic stem cells (HSC) as means to genetically transfer HIV protective genes into subjects with HIV infection. Taken as a group, these trials have shown the safety of both the procedure and the anti-HIV agents themselves and the feasibility of the approach. They point to the requirement for the ability to transduce and infuse as many gene containing cells as possible and/or effecting a high level of engraftment and in vivo expansion of these cells and the need for increased efficacy of the anti-HIV agent(s) and the importance of selecting the proper patient population for investigation. While the field is still in its early phase of development and clinical investigation, the remarkable single case of the “Berlin patient” who achieved a functional cure without evidence of remaining HIV-1 after receiving an allogeneic HSC transplant of CCR5 delta-32 homozygous donor cells makes the possibility of achieving this goal a distinct possibility.
Stem Cell Gene Therapy for HIV with Multiplexed RNA: Process and Clinical Development Towards a Cure for HIV

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Stem cell gene therapy for HIV is a promising alternative to lifelong drug treatment and our laboratories have developed multiplexed, RNA-based HIV inhibitor strategies to treat this disease. Between 2006 and 2008, we performed a pilot study in HIV+ patients undergoing autologous transplantation for lymphoma in which we transplanted gene modified (HIV-resistant) hematopoietic stem and progenitor cells (HSPC) following fully ablative patient conditioning. We observed gene marking in 4/4 patients for up to 6 months and long-term gene marking and expression (>3 years) in peripheral blood and bone marrow cells in one patient. Structured treatment interruption in the latter patient resulted in a rapid and significant rebound of viral load, followed by a slight increase in gene-modified peripheral blood cells once cART therapy was reinitiated. Subsequently, we have developed a second generation of lentiviral vectors that have an improved safety profile, a drug resistance gene and potent HIV activity, with up to 5-logs reduction of virus production in cell lines and primary cells challenged in vitro. We have also improved methodologies for transduction of HSPC and developed an immunodeficient animal model of HSPC transplant and HIV challenge. This model system has been used to evaluate ex-vivo manipulation of HSPC, in vivo drug enrichment of gene modified cells and to screen candidate vectors for their relative protection against in vivo HIV challenge. Improved methods of gene transduction result in a significantly increased frequency of gene modified CD4+ cells in vivo as does in vivo drug selection. In vivo HIV challenge results confirm that our first generation vector expressing multiple anti-HIV RNAs provides a selective survival advantage to gene modified CD4+ cells and thus validates the use of this pre-clinical model for testing our second generation vectors. Based on our results in these and other studies, we have designed a series of clinical trials to test our new vectors in the context of non-ablative conditioning in lymphoma patients and non-malignant patients with poor immune recovery on cART. Updated clinical results and scientific progress from these studies will be presented.
Bryan Burke, PhD
Associate Director, Clinical Research & Development, Calimmune

Engineering Cellular Resistance to HIV-1 With a Dual Therapeutic Lentiviral Vector

BP Burke¹, BR Levin², J Zhang¹, MV Carroll¹, JC Colón¹, N Keech¹, V Rezek², G Bristol², MP Boyd¹, SG Kim², DN Vatakis², LR Breton¹, JS Bartlett¹, SG Kitchen² and GP Symonds¹

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Background: Transplantation of CCR5-/- hematopoietic stem cells has recently 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 combinatorial approach has two points of inhibition for R5-tropic HIV-1 and is also active against X4-tropic strains of HIV-1 that do not use CCR5 as a coreceptor.

Methods: We have utilized the humanized bone marrow, liver, thymus (BLT) mouse model to characterize the ability of LVsh5/C46 to confer protection from HIV-1 pathogenesis. Two groups of BLT mice were generated from the same donor tissue and tested in parallel. A control group received non-modified human CD34+ hematopoietic stem/progenitor cells (HSPC), while the treatment group was transplanted with human CD34+ HSPC transduced with LVsh5/C46. Hematopoietic engraftment and susceptibility to HIV-1 infection was compared between the two groups of animals.

Results: The treatment group was transplanted with human CD34+ HSPC containing 3 LVsh5/C46 vector copies per cell, and displayed no significant difference in hematopoietic engraftment and multi-lineage differentiation compared to the control group, which included robust CD4+ T-cell development. Splenocytes isolated from the treatment group were resistant to both R5- and X4-tropic HIV-1 during ex vivo challenge experiments. In vivo challenge with R5-tropic HIV-1 displayed significant protection of CD4+ T-cells and reduced viral load within peripheral blood and lymphoid tissues of the treatment group up to 14 weeks post-infection. LVsh5/C46 gene-marking and transgene expression was confirmed stable at 26 weeks post-transplantation.

Conclusion: These pre-clinical data strongly support the use of LVsh5/C46 lentiviral vector in gene and cell therapeutic applications for inhibition of HIV-1 infection.
Engineering Stem Cells With Chimeric Antigen Receptors For HIV-Specific T Cell Immunity

A Zhen, V Rezek, J Rick, S Kasparian, B Levin, M Kamata, ISY Chen, JA Zack, SG Kitchen

David Geffen School of Medicine at UCLA

The HIV-specific cytotoxic T lymphocyte (CTL) response is a critical component in controlling HIV replication. We are interested in the development of ways to genetically enhance the HIV-specific CTL response to allow long-term viral suppression or viral clearance. We have previously demonstrated that human hematopoietic stem cells (HSCs) can be modified with a molecularly cloned HIV-specific T cell receptor (TCR) to develop into fully functional CTL that can suppress HIV replication in a humanized mouse model. A fundamental issue with this approach is the notion that these TCRs are human leukocyte antigen (HLA)-restricted, and therefore their use therapeutically is highly restricted to individuals with specific HLA genotypes.

We are currently investigating the use of non-HLA restricted chimeric antigen receptors (CARs) that allow the recognition of HIV when expressed by a CTL. Here we report that the use of a CD4-ζ chain CAR that contains the extracellular portion of the CD4 molecule fused to the intracellular TCR-zeta signaling domain. The lentiviral vector containing the CD4-ζ CAR also expresses small hairpin (sh)RNAs specific for CCR5 and the HIV LTR to protect the developing cells from infection. We determined that CD4-ζ CAR transduced HSCs can differentiate into functional CD4 and CD8 T cells as well as NK cells in vivo in humanized mice. Importantly, we found that CD4-ζ containing cells can functionally respond to HIV over long periods of time and significantly suppress HIV replication following infection. Thus, this system allows the close examination of the engineering of antiviral immunity and non-HLA restricted HIV-specific CTL responses in vivo. Our results strongly suggest that stem cell based gene therapy may be a feasible approach in the treatment of chronic viral infections and provide a foundation towards the development of this type of strategy.
Towards in vivo genome engineering of hematopoietic stem cells for HIV therapy

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**Background:** Our goal is to achieve long-term CCR5 knockout in hematopoietic stem cells (HSCs) by a simple intravenous injection in order to protect high-risk groups from HIV infection. Targeting HSCs rather than CD4+ T-cells has the advantage that all potential HIV target tissues can be protected. Towards our goal, we are in the process of developing an in vivo HSC genome editing approach. This approach involves the clinically well established and relatively inexpensive procedure for HSC mobilization by G-CSF/AMD3100 followed by an intravenous injection of a HSC-targeting helper-dependent adenovirus Ad5/35 vector expressing a CCR5-specific ZFN (HD-Ad5/35.ZFN).

**Methods/Results:** We studied in vivo transduction of mouse HSCs (LSK cells) in human CD46-transgenic mice and human HSCs in the CD34+/NOG model. We showed that mobilized HSCs are efficiently infected by intravenously injected Ad5/35 vectors expressing GFP. More than 20% of mobilized HSCs are transduced in peripheral blood. At day 3 post-Ad injection, about 9% and 13% of HSCs in the bone marrow and spleen, respectively, expressed GFP. This means that 0.01% of bone marrow cells were transduced HSCs.

To produce the HD-Ad5/35.ZFN we developed a miRNA-regulated expression system that would suppress ZFN expression in Ad5/35 producer cells (293 cells) but allow it in HSCs (CD34+ cells). After ex vivo transduction of CD34+ cells with HD-Ad5/35.ZFN, we demonstrated stable ccr5 knockout in primitive HSCs, i.e. long-term culture-initiating cells (LT-CIC) and SCID-NOD repopulating cells. Studies on in vivo HSC transduction with HD-Ad5/35-ZFN are ongoing.

**Conclusions:** Our simple procedure of in vivo genome editing of HSCs has major advantages over currently used approaches that involve the collection of HSCs from patients, their in vitro culture/transduction, and retransplantation into myelo-conditioned patients. Because CCR5 plays a dominant role in the initial HIV infection, knockout of this receptor might have a prophylactic effect. Although initially only a fraction of HSCs will be gene-modified and only a fraction of HSC progeny will be protected, upon HIV infection, this fraction will selectively expand, mount an active immune response against the virus and rescue the patient.
SESSION 7 SPEAKERS

Jennifer E. Adair, PhD
Associate in Clinical Research, Clinical Research Division, Fred Hutchinson Cancer Research Center

Novel Integrated Hematopoietic Clone Tracking in Nonhuman Primates Suggests a Minimal Population of Long-term Repopulating Clones in CD34-Enriched Cell Pools

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While the total number of allogeneic cells required to reliably reconstitute hematopoiesis following hematopoietic stem cell transplantation (HSCT) has been established, we know very little about the actual number or characteristics of true long-term, multipotential autologous HSCs, critical for any gene therapy, gene editing or cord blood transplantation approach. Integration site (IS) analysis in gene therapy trials using integrating retroviral vectors (RV) and lentiviral vectors (LV), suggest very few transplanted clones contribute, but underestimates clonal diversity due to procedural bias. DNA barcoding via RV tagging has successfully quantitated hematopoietic reconstitution in mice, but relevance to human hematopoiesis is limited and DNA barcoded vector libraries reported to date lack complexity to recapitulate quantitative tracking in clinically-relevant large animal models. Moreover, permanent integration of proviral DNA into the cell genome is not a benign molecular event and can influence reconstitution kinetics. We hypothesized that IS analysis and DNA barcoding together would provide more robust HSCT reconstitution data. We developed a high complexity (~1.2 million), DNA barcoded, HIV-1-based LV library encoding the chemotherapy resistance gene MGMT(P140K) and enhanced green fluorescent protein (GFP) to measure hematopoietic reconstitution following autologous HSCT in pigtailed macaques. A total of 3.6E+06 autologous CD34+ cells (3 x library complexity) were transduced at a MOI of 20 (10 x 2) and infused into two animals following myeloablative total body irradiation. We observed ~2 and 12% GFP+ peripheral blood white blood cells (PB WBCs) following hematopoietic recovery in these animals, respectively. We performed barcode and IS retrieval from PB WBCs collected early (1 month) after HSCT and observed <0.001% of infused clones contributing to hematopoiesis by DNA barcoding, and ~42% fewer clones via MGS-PCR of the same sample, with similar contributions of the most dominant clones identified by each method. Over 1 year following HSCT, a total of 5,089 (0.4%) and 20,799 (1.7%) infused clones contributed to hematopoiesis in each animal, respectively, with polyclonal contribution patterns. At 1 year post-transplant, we analyzed PB WBC subsets and identified a total of 116 and 2,246 clones respectively contributing to each of three lineages (T, B, and granulocyte), suggesting <0.2% of infused CD34+ cells displayed multi-lineage potential. Adjusting for marking levels we estimate between 5000-20,000 clones gave rise to trilineage hematopoiesis at 1 year post-HSCT. These findings underscore the need to quantitatively assess repopulation kinetics of HSCs under various conditions aimed at increasing gene modified cell engraftment, especially in patients with candidate diseases for gene editing strategies, such as HIV. Here we provide a tool by which to comprehensively analyze efficacy and safety of approaches such as HSC expansion or MGMT(P140K)-mediated in vivo chemoselection, and a baseline for comparison and evaluation in the clinically-relevant nonhuman primate model.
A Novel Anti-HIV Chimeric Antigen Receptor: Toward a Functional Cure

Edward Berger, PhD  INVITED SPEAKER
Chief, Molecular Structure Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

SESSION 7 SPEAKERS

Edward Berger, PhD  INVITED SPEAKER
Chief, Molecular Structure Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health

A Novel Anti-HIV Chimeric Antigen Receptor: Toward a Functional Cure

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Background: While HAART efficiently suppresses HIV replication below detectable levels, these drugs do not kill cells that are already infected. Therefore targeted cell killing represents a conceptually powerful complement to HAART for achieving a cure. Indeed we previously showed in a murine acute HIV-1 infection model that the synergistic combination of HAART plus recombinant immunotoxins (RITs) targeting the HIV-1 Env glycoprotein potently blocks virus rebound after treatment cessation (Goldstein et al. 2000). However in a murine model of chronic infection, RIT augmentation of HAART promoted further suppression of viral loads in multiple tissues but did not eliminate the virus (Denton et al. 2014). Therefore, while an RIT plus HAART might help cure acute infection, a durable (lifelong) version of targeted cell killing is presumably required for chronic infection.

Methods: Encouraged by recent successes against cancer with adoptive transfer of genetically modified T cells, we have designed novel bispecific chimeric antigen receptors (CARs) for durable killing of HIV-infected cells. As a targeting domain for the CAR, we attached human CD4 (D1D2) by flexible polypeptide linkers to an scFv of human mAb 17b against the highly conserved coreceptor binding site on gp120. We compared the anti-HIV activities of T cells transduced with the CD4-17b CARs versus a “standard” CD4 CAR containing the CD4 moiety only.

Results: Compared to a standard CD4 CAR, the CD4-17b CAR showed enhanced suppression of replication for genetically diverse HIV-1 isolates. Analyses of CD4-17b CARs possessing different linker lengths suggested the importance of serial triggering for potent virus suppression activity. Importantly, the CD4-17b CARs were completely devoid of a highly undesired activity observed with the standard CD4 CAR, i.e. rendering the transduced CD8 T cells susceptible to HIV-1 infection.

Conclusion: The bispecific CD4-17b CAR provides several important advantages compared to standard CD4 CAR. The minimal immunogenicity of these CARs, coupled with their MHC-independent activity and targeting of highly conserved immutable sites on Env, highlight the advantages of CARs over TCRs for genetic modification of T cells against HIV. The CD4-17b CARs offer considerable promise in the quest for a functional cure of HIV infection.
Rational Chimeric Antigen Receptor Design Towards Long-Term Control of HIV Infection

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Today, an HIV-infected individual may be successfully treated using combination antiretroviral therapy. While efficacious, these drugs are not curative, and accumulate toxicity over a patient’s lifetime. Furthermore, the establishment of a persistent latent reservoir during the course of infection complicates cure strategies using replication inhibitors alone. To better target the latent reservoir to potentially allow long-term cessation of antiretroviral therapy, we developed and tested several novel chimeric antigen receptors (CARs) with enhanced antiviral activity. CARs consisting of an extracellular targeting domain linked to second generation signaling motifs developed in the cancer field have recently shown promising results as therapeutic agents for hematologic malignancies. In addition, CAR-expressing T-cells have been shown to persist for extended periods of time, and to traffic extensively throughout the tissues—features that are attractive for attacking the latent reservoir. We found that CD4-based CARs are specifically reactive to HIV-Envelope glycoprotein gp120, and are triggered even by profoundly low levels of antigen. Moreover, by linking CD4 to the CD4i Ab 17b, we were able to achieve enhanced potency in suppressing spreading infection in PBMCs of a diverse panel of primary HIV-1 isolates. Interestingly, this observation was only seen with a CD4-17b CAR variant where the linker between CD4 and 17b was too short to enable simultaneous binding. Our findings are consistent with the serial triggering model for T-cell activation, whereby too-high affinity for antigen is detrimental to cell-mediated immunity. Finally, we observed that CD8 T-cells expressing CD4 alone as the CAR ectodomain became highly permissive to viral infection, whereas the CD4-17b CAR was completely resistant to CAR-mediated entry by virtue of steric hindrance of the 17b moiety. We propose that our short-linker CD4-17b CAR is a promising and complementary agent to strategies seeking a functional cure for HIV infection.
Fadila Bouamr, PhD  
*Chief, Viral Budding Unit, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health*

**Specific Delivery of a Deubiquitinase to HIV-1 Assembly and Budding Sites Halts Virus Production**

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In order to spread infection, HIV-1 as well as most enveloped viruses utilize host proteins to catalyze membrane fission and escape cells. The host proteins involved in these processes are members of the endosomal sorting complex required for transport (ESCRT), a cellular machinery that coats the inside of viral budding necks to perform membrane modeling events necessary for virus abscission. Detailed understanding of how HIV-1 utilizes host proteins to leave the cell at the molecular level aims at the identification of new targets and strategies to inhibit virus production. This is best exemplified with studies on the role of ubiquitin in ESCRT-mediated virus budding, which clearly supported a pivotal role. However, direct evidence for ubiquitin in HIV-1 escape from cells remained elusive. We recently demonstrated a direct and natural role of ubiquitin conjugation in HIV-1 production using a new approach, involving the strategic and specific delivery of a deubiquinase to viral assembly and budding sites. We fused the catalytic domain of the Herpes Simplex Virus UL36 (DUb) to either HIV-1 Gag or the Gag-binding ESCRT-component TSG101. DUb expression vectors harboring Gag in fusion with DUb (Gag-DUb) were designed to incorporate DUb activity early in HIV-1 genesis and during Gag assembly. DUb-TSG101 vectors target late events of HIV-1 genesis and incorporate DUb into viral budding necks, TSG101 natural sites of virus membrane fusion and abscission. We observed a strong and specific inhibition of HIV-1 production with both types of vectors; that led to the accumulation of HIV-1 budding particles at the plasma membrane and a severe decrease in the production of infectious virions (less than 5%). In contrast equine and murine retroviruses retain virus production in identical conditions demonstrating that targeting is specific and confined to HIV-1 sites of production from the plasma membrane. These findings demonstrated that delivery of DUb to sites of HIV-1 genesis is a novel and powerful approach to halt virus production. Specific lentiviral-based delivery vectors of DUb are in progress. Also insertion of DUb-fusion sequences to engineer CD4+ T cells virtually incapable of producing HIV-1 virions will be discussed.
Hans-Peter Kiem, MD
Co-PI, defeatHIV
Co-Chair, Program in Transplantation Biology, Full Member, Clinical Research Division, Fred Hutchinson Cancer Research Center

Hematopoietic Stem Cell Gene Therapy for HIV: From Preclinical to Clinical Studies

HP Kiem
Fred Hutchinson Cancer Research Center and University of Washington

The combination of genetic modification and hematopoietic stem cell transplantation may provide the necessary means to develop an alternative treatment option to conventional antiretroviral therapy. As hematopoietic stem cells give rise to all hematopoietic cell types susceptible to HIV infection, modification of hematopoietic stem cells is an ideal strategy for the development of infection-resistant immune cell populations. Although promising results have been obtained in multiple animal models, additional evidence is needed to convincingly demonstrate the feasibility of this approach as a treatment of HIV-1 infected patients. So far the Berlin Patient remains the only patient cured of HIV. This was accomplished after receiving an allogeneic cell transplant from a donor who was homozygous for CCR5 delta32 and thus resistant to HIV infection. Many questions remain as to what contributed to the success of this transplant. We have studied autologous transplantation of genetically modified and HIV-resistant hematopoietic stem cells in preclinical models of HIV/AIDS. We will discuss our results in a nonhuman primate model using the anti-fusion peptide mC46 and other anti-HIV strategies. We will also discuss the impact of these studies on the design of clinical trials for both autologous and allogeneic transplantation.
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Reactivation of latent HIV-1 by type I interferon agonists is regulated by RNA binding proteins

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Background: To contribute to a functional cure for HIV infection, we are investigating innate immune pathways to target and eradicate latent HIV reservoirs. Type I interferon (IFN-β/α) pathways are triggered when cellular receptors recognize viral pathogen-associated molecular patterns (PAMP) contained in viral RNA or DNA. Interferon-stimulated genes (ISG) subsequently function to restrict virus replication. In studies of RNA viruses, we identified novel RNA binding proteins that regulate interferon responses that also have been implicated in the HIV-1 life cycle. Moreover, proteins involved in transcription from the HIV-1 LTR promoter, including nuclear factor kappa-B (NF-kB) and chromatin modifying complexes, intersect with expression of IFN-β. We hypothesized that by inducing type I IFN, a transcriptional cascade will occur leading to expression of latent HIV.

Methods: To test this hypothesis, we transfected J-Lat H2 cells, a CD4+ T cell line containing an integrated HIV-1 sequence including 5’LTR and a GFP reporter gene, with type I IFN agonists: short chain poly-I:C, long chain poly-I:C, and Sendai virus RNA, compared to latency-reactivating drugs SAHA (a chromatin modifier) and prostratin (NF-kB activator). Human RNA binding proteins were targeted by RNA interference (mRNA depletion).

Results: In J-Lat cells, analysis of GFP reporter expression indicated HIV-1 transcription was activated by viral RNA PAMP, with long chain poly-I:C giving the greatest response. In preliminary experiments, siRNA knockdown of RNA binding proteins NF90, hnRNPA1, and RNaseH2 exhibited a 2- to 5-fold increase in HIV-1 transcription in cells transfected with PIC-H or Sendai virus RNA. Knockdown of NF90 also enhanced HIV-1 reporter expression by prostratin or SAHA approximately 2-fold.

Conclusion: While not as effective as prostratin or SAHA, type I IFN agonists are capable of inducing expression of latent HIV-1 in CD4+ reporter cells. Novel negative regulators of HIV-1 reactivation by viral RNA PAMP include cellular RNA binding proteins NF90, hnRNPA1, and RNaseH2. NF90 also negatively regulates HIV-1 reactivation by SAHA and prostratin, suggesting a general repressive mechanism on HIV-1 gene expression.

Mechanisms how type I IFN pathways induce reactivation of latent HIV-1 are under investigation, to contribute to the development of new antiviral therapies to eradicate latent HIV reservoirs.
Impact of Epigenetic Modification on Rare-Cutting Endonuclease-Mediated Mutagenesis Efficiency Against Viral Genomes

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Our laboratory is developing a new therapeutic approach to cure persistent/chronic viruses, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and herpes simplex virus (HSV). In this therapeutic approach engineered rare-cutting endonucleases are used to selectively target, cleave, and disrupt viral sequences within persistently/chronically-infected cells. The ability of an endonuclease to specifically target and disrupt latent viral sequences relies on highly specific DNA binding activity that is followed by the introduction of DNA double strand breaks (DSBs), and subsequent repair by the error-prone non-homologous end joining (NHEJ) repair pathway.

The efficiency of endonuclease binding activity to its target sequence may be influenced by the DNA structure and accessibility. The activity of four zinc finger nucleases directed towards HIV sequences was shown to vary dramatically depending on whether the targeted sequences were present in a plasmid or an integrated provirus. Epigenetic modifications such as histone tail modifications and DNA methylation can dictate chromatin structure and accessibility, and have been reported to influence endonuclease-mediated cellular genome editing. Therefore, we investigated whether the efficiency of targeted mutagenesis of distinct viral genomic sequences could be modulated using chromatin modifiers such as histone deacetylase inhibitors (HDACi). We performed a proof of concept study in an in vitro model of latent herpes simplex virus (HSV) infection. Published studies have shown that viral gene expression during latent HSV infection is repressed by viral genome chromatinization and histone tail modifications similar to those of heterochromatin or transcriptionally inactive DNA. Our data showed that following exposure to an HSV-specific rare-cutting endonuclease, targeted mutagenesis of HSV genomes is increased by 2 to 6 fold in HSV-infected cells treated with HDACi compare to infected cells left untreated. This suggested that targeted DNA accessibility to endonuclease can be modified using HDACi. The option of using HDACi or other chromatin modifiers to increase the efficiency of endonuclease-mediated mutagenesis may be of broad utility for targeted gene disruption, including that of viral genomes.
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Optimization of mRNA for Therapeutic Use

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Introduction: RNA-based therapeutics are more suitable than other gene-based therapies for applications requiring delivery with non-viral carriers, targeting, transient expression, or absence of gene disruption/integration into the genome. Dozens of small RNA-based therapeutics including siRNAs and aptamers have reached clinical and preclinical testing, illustrating non-viral RNA delivery practicability. However, protein encoding mRNA delivery presents new challenges, including mRNA stability, efficient protein translation, and pro-inflammatory response minimization. Here, we investigated template modification effects on mRNA translation and gene modifying enzyme mRNA delivery for HIV co-receptor and viral gene excision is under investigation.

Methods: Various mRNAs were modified with 2’-fluoro (2’F) substitutions or addition of 5’/3’ noncoding sequences via in vitro transcription. Modified mRNAs encoding eGFP, cas9 and Cre recombinase were assayed for translational capability in cells. HEK293 cells and CHO cells were transfected with eGFP mRNA and TransIT-mRNA (Mirus Bio) followed by protein analysis. Cre and cas9 (co-transfected with loxp targeting gRNA) mRNA were transfected in HT1080 cells integrated with UbC-LoxP-CFP-LoxP-TagRFP lentiviral vector. Effective protein production results in excision of CFP and concurrent RFP expression, measured using flow cytometry. mRNA stability was measured with quantitative PCR (qPCR) and cellular protein duration analysis. Based upon our in vitro results, we have begun testing in vivo potential for modifications using cas9 and cre mRNA systems in cre/loxp mice.

Results: Nucleotide substitution of ATP with 2’F-ATP results in levels of translation similar to unmodified transcripts, and mRNA stability is improved. In addition, addition of noncoding sequence before or after the poly(A) tail has minimal effect on translation. We are currently investigating effects of sequence additions on stability and on in vivo inflammatory response. Cre mRNA successfully induces a color switch in vitro and in vivo, providing an example of gene editing in an in vivo model.

Conclusions: Chemical and structural modifications of mRNA are necessary for improving its properties for therapeutic use. Our study reveals that such modifications may enhance mRNA stability and non-immunogenicity without compromising mRNA translational capacity. Moreover, we continue to evaluate better, more targeted delivery systems relative to conventional delivery modes, including lipid-based transfection (TransIT-mRNA, Mirus), PAMAM dendrimer, or electroporation approaches.
POSTERS

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Targeted Disruption of Essential HIV Proviral Genes by Zinc Finger Nucleases

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Despite the enormous success of Highly Active Antiviral Therapy (HAART) in suppressing viremia of HIV-infected individuals to nearly undetectable levels, HAART is not able to eliminate the latent viral reservoir that is present in long lived central memory T cells (Tcm). Latency in Tcm allows HIV to remain transcriptionally silent evading both HAART and immune detection. However latency is a reversible state, and upon activation of the latently infected cell the provirus serves as a template for the production of new progeny virus. Consequently, all infected individuals must remain on therapy throughout their lifetime due to the inevitable viral rebound that occurs within weeks of interruption of suppressive HAART or be at risk of chronic infection and developing AIDS. In recent years, curative HIV therapy that eliminates or sterilizes cells that are latently infected with HIV has been proposed. One such proposed therapy is the targeted disruption of essential genes within the provirus in order to prevent production of viable progeny upon reactivation. Here we show that HIV-specific zinc finger nucleases (ZFNs) targeting the HIV pol gene can be used to efficiently disrupt the integrated HIV provirus. By using self complimentary adeno-associated virus vectors (scAAV) to deliver ZFNs into SupT1 cells infected with a defective HIV (DHIV) we were able to disrupt reverse transcriptase (RT) and integrase sequences in up to 28% of DHIV proviruses. Consistent with previous reports, upon treatment with the end processing exonuclease Trex2 the frequency of ZFN-mediated targeted disruption within reverse transcriptase and integrase was increased up to two fold. Two additional ZFN pairs targeting Protease and RT efficiently plasmid derived target sequences. However, they were not able to cleave an integrated provirus suggesting that not all regions of the integrated provirus maybe accessible to cleavage by targeted endonucleases. These data demonstrate the potential of targeted gene disruption as a curative therapy for latent HIV infections.
Alison Dewey

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Safety and Delivery of Rare-Cutting Endonucleases in Vivo for Treatment of Persistent Viral Infections

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Background: Antiviral treatments for most persistent viral infections do not affect latent viral reservoirs of viral DNA, and only minimize the symptoms of virus infection for the duration of treatment. A possible curative approach targets essential viral genes with DNA cleavage enzymes to introduce mutations that inhibit virus replication. This method has been demonstrated in vitro for herpes simplex virus, hepatitis B virus, and human immunodeficiency virus (HIV). However, the safety of this approach and the feasibility of delivering enzymes to relevant cell reservoirs harboring latent viral genomes in vivo have not been established.

Methods: In the context of HIV infection, we have examined the safety of HIV-specific zinc finger nucleases (ZFNs) in vitro by treating SupT1 cells with vectors expressing four different ZFNs, and measured cytotoxicity by PI and AnnexinV staining after 4 and 7 days. In order to investigate the practicality of delivering enzymes to a cellular reservoir of HIV, we evaluated a panel of Adeno-Associated Virus (AAV) vectors for transduction of CD4 T cells in vitro. Transduction levels were measured in the SupT1 CD4+ T cell line, as well as in primary human central memory T cells.

Results: In vitro data shows that by PI and AnnexinV staining HIV-specific ZFNs are not toxic to SupT1 cells at either time point, and that AAV 1, 4, and 6 vectors can efficiently transduce SupT1 cells and primary human central memory T cells. Data from ongoing in vivo endonuclease safety studies will also be presented.

Conclusion: Our data indicates that safety and delivery of DNA cleavage enzymes should be investigated further in vivo. Accordingly, we will examine safety in vivo in healthy C57BL/6J mice treated with endonucleases targeting mouse genes. After treatment, we will assess blood chemistry, white blood cell counts, and histology for treated and control groups throughout their natural lifespan (18 months). Furthermore, we plan to investigate in vivo vector delivery to lymphocytes using humanized NOD/scid/IL2 receptor gamma chain knockout (NSG) mice.
HLA-F and MHC Open Conformers in a Novel HIV-1 Immunization Strategy

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HLA-F is expressed as a protein independent of bound peptide or β2-microglobulin and surface expression is upregulated in dendritic cells, monocytes and most lymphocyte subsets upon activation. Classical MHC class I (MHCI) is also expressed on proliferating lymphoid cells as so-called ‘open conformers (OCs)’, in addition to the ubiquitously expressed form complexed with peptide and β2-microglobulin. Our studies showed that HLA-F binds most MHCI proteins as open conformers without peptide but not as peptide bound complex. These studies were further extended to show that both HLA-F and MHCI OC are ligands for a specific subset of killer Ig-like receptors (KIRs), defining a new paradigm for MHCI function and communication between the innate and acquired immune responses. The HLA-F/MHCI physical interaction was further implicated in the function of HLA-F and MHCI open conformers in a general mode of exogenous MHCI antigen uptake and antigen presentation by activated immune cells that differs from the canonical MHCI endogenous antigen presentation. A long-term extension is to dissect the HLA-F/MHCI OC pathway to uncover the requirements for antigens to access the pathway. We are currently testing the hypothesis that antigen entry is governed by a synergism between specific structural characteristics of the exogenous antigen and the MHCI allele types of target cells. New evidence is presented using HIV-1 gag p24 derived 50 amino acid polypeptides suggesting a requirement for HLA class I peptide epitope specificity in antigen uptake, upstream of antigen presentation. These experiments suggested that the physical proximity of HIV-1 class I and class II peptide epitopes within an extended polypeptide can govern antigen presentation of epitopes within the same polypeptide, through either MHCI or MHCII. A goal is to manipulate these features in designing effective immunogens for directed stimulation of antigen-specific host responses.
**Tiffany Hensley-McBain**  
Graduate Student, Molecular and Cellular Biology Graduate Program, University of Washington

**Dysfunctional Neutrophil Responses to SIV Infection**

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**Background:** Recent studies indicate that individuals with low neutrophils (PMN) are at increased risk of HIV infection. Also, the RV144 vaccine trial implicated non-neutralizing antibodies and associated Fc-mediated functions in vaccine-induced protection. These studies suggest that early innate antiviral functions and Fc-mediated functions of PMN may play an important role in mediating early viral control, yet the role of innate cellular responses in preventing the establishment of the viral reservoir remains unknown. The potential to contribute to the early control of viral spread may depend on the kinetics of PMN mobilization, activation, and recruitment during acute HIV/SIV infection.

**Methods:** We assessed kinetic changes in PMNs in peripheral and mucosal tissues during acute SIV infection in six rhesus macaques challenged i.r. with 100,000 TCID50 of SIVmac239X. Flow cytometry, CBC, and luminex were used to assess PMN and cytokine levels and related PMN functional markers. Samples were collected pre-SIV and days 3, 7, 14, 21, 28, 42, and 63 post-SIV.

**Results:** We observed a significant decrease in systemic IL-17 (p=.0313) and a trending decrease in G-CSF (p=.0625) early after SIV. Surprisingly, blood PMN concentrations steadily decreased after infection, and no significant increase of PMN was detected in gut or lymphoid tissues. In addition, blood PMN numbers and rectal PMN percentages significantly correlated (p=.0032). Lastly, HLA-DR (p=.0313), CD86 (p=.0156), and FcγRI (p=.0313) were significantly upregulated on PMN during acute SIV infection, suggesting PMN activation without concurrent tissue recruitment as expected during acute viral infection.

**Conclusions:** In contrast to other acute viral infection models, PMN-supporting cytokines are decreased or not induced during acute SIV, potentially contributing to lack of PMN mobilization and recruitment to the tissues. Further, blood and rectum PMN levels correlate post-SIV, suggesting that blood PMN concentration may directly impact recruitment to gut tissues. Lastly, upregulation of markers involved in antigen presentation and Fc-mediated functions highlights the potential diverse functional roles of neutrophils during acute SIV infection and the mechanisms by which neutrophils, if induced, could contribute to more effective viral control. However, lack of neutrophil induction during acute SIV may contribute to the establishment of the viral reservoir.
POSTERS

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Cocal Envelope is Superior to VSV-G for Lentiviral Gene Transfer of Human and Primate HSCs and T Cells

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Lentiviral vectors are routinely used for stable gene transfer and are showing great promise for clinical applications. In particular, efficient gene modification of hematopoietic stem cells and T cells lays the foundation for the therapeutic treatment of several human diseases such as cancer and HIV. Lentiviral vectors are commonly pseudotyped with vesicular stomatitis virus envelope glycoprotein (VSV-G) with the advantages of conferring broad tropism to the vector and allowing concentration by centrifugation. However, the use of VSV-G also comes with several drawbacks, such as its susceptibility to inactivation by human serum complement and its toxicity when constitutively expressed, which has impeded efforts to generate packaging cell lines. Here, we show data suggesting that the cocal envelope offers a superior alternative to VSV-G for pseudotyping lentiviral vectors. Cocal lentiviral vectors significantly increased gene transfer of both human and pigtail macaque (Macaca nemestrina) CD34+ cells as compared to VSV-G vectors. Plating efficiency was similar for VSV-G and cocal modified human CD34+ cells, and differentiation as determined by CFU assay was not skewed. Cocal-pseudotyped lentiviral vectors also transduced human CD3+ more efficiently than VSV-G-pseudotyped vectors and showed comparable gene transfer in pigtail macaque CD3+ cells. In addition, our previous finding showed that cocal is less prone to human serum inactivation than VSV-G, and may thus be better suited for in vivo delivery of the vector.

As an initial step toward the generation of a packaging cell line, we stably expressed either the VSV-G or cocal envelope in 293T cells under the control of a CMV promoter. Cocal expressing cells consistently produced half a log to a full log more infectious particles as compared to VSV-G expressing cells. Overall, the cocal envelope may prove to be the preferred choice for gene transfer in HSCs and T cells, for in vivo delivery of the vector, and for the making of a packaging cell line.
Paul Munson
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A Conserved Elements (CE) DNA Vaccine Induces T-cell Responses in the Setting of Pre-existing Immunodominant Responses Induced by Chronic Viral Infection

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Introduction: We have previously shown that in SIV-infected rhesus macaques undergoing antiretroviral therapy (ART), DNA immunization protected ~50% of animals from viral rebound after discontinuing ART. To improve this approach, we are investigating a conserved elements (CE) therapeutic DNA vaccine that consists of conserved regions of SIV. However, it is unknown whether a vaccine expressing conserved, but generally subdominant epitopes, can induce responses against CE in the setting of an immunodominant response induced by infection.

Methods: Two groups of rhesus macaques chronically infected with SHIV89.6P but maintaining persistent low to moderate viral loads were vaccinated with either a full-length Gag or CE DNA vaccine. Immune responses were measured by IFN-\(\gamma\) ELISpot and ICS.

Results: Prior to immunization, both groups had similar responses to Gag (\(p = 0.56\)) with little detectable responses to CE as determined by IFN-\(\gamma\) ELISpot. The Gag vaccine group exhibited a two-fold decrease in CE-specific responses relative to pre-immunization responses. In contrast, CE vaccinated animals developed a cumulative ten-fold increase in IFN-\(\gamma\) T-cell responses against multiple CE sequences. Furthermore, CE vaccinated animals had a higher magnitude of poly-functional responses to conserved regions relative to SIV Gag vaccinated animals.

Conclusions: These results illustrate that a CE DNA vaccine during chronic viral infection can increase immune targeting to CE. These results support the feasibility of developing a therapeutic CE DNA vaccine to induce a functional cure against AIDS.
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Targeted DNA Mutagenesis Using Engineered Meganucleases as a Potential Cure For HIV

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Background: In 2012 less than 35% of HIV positive patients (9.7 million) that were eligible for highly active antiretroviral therapy (HAART) were receiving it. HAART delays progression to AIDS but does not cure the infection so patients have to take lifelong antiretroviral therapy. The major barrier to HIV cure is the presence of a long-lived latent viral reservoir. This latent viral reservoir is inaccessible to HAART and persists for the lifetime of the patient. To target the integrated HIV provirus in latently cells, we are using engineered endonucleases specific to the HIV pol gene. These endonucleases consist of an engineered meganuclease that has been linked to a transcription activator-like effector (TALE) domain creating a fusion enzyme that is called a megaTAL. MegaTALs have improved target recognition, higher binding specificity and reduced off target cleavage effects. MegaTALs have about a 20-fold increased activity compared to standalone meganucleases (Boissel et al., 2013). When megaTALs bind to a target DNA sequence, they introduce double stranded DNA (dsDNA) breaks. The dsDNA breaks are repaired by non-homologous end joining (NHEJ), which is error-prone and may introduce mutations in the target sequence. We have coupled the megaTALs with Trex2 exonuclease that processes the 3’-overhangs created when the megaTALs resect the dsDNA. Trex2 when coupled with a meganuclease increases the chances of dsDNA break repair by NHEJ and leads to a five-fold increase in disruptive mutations in target genes; and therefore of introducing mutations in the target HIV sequence (Certo et al., 2012). We hypothesize that if we can introduce disruptive mutations in an essential gene in the integrated HIV provirus, we can prevent HIV reactivation. Inhibition of reactivation would create a functional cure of HIV.

Methods and Results: We have delivered megaTALs to CD4+ supT1 cells containing an integrated HIV provirus using lentiviral vectors. We can express megaTALs in CD4+ supT1 cells up to 70-90%. Our results show that we can introduce mutations in our target sequence in the integrase coding region of the integrated HIV provirus. We are able to introduce mutations in 8% of our target HIV sequences. We have optimized a physiological model of HIV latency and are currently testing HIV-specific megaTALs for their ability to inhibit HIV reactivation from latency.

Conclusion: Targeted mutagenesis of the integrated HIV provirus could provide a potential functional cure of HIV disease and eliminate lifelong dependence on HAART.
Biswajit Paul
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Developing More Efficient Protective Therapies Against HIV: Combining MegaTAL Nuclease Driven Genome Engineering and Chemoselection

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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 demonstrate (a) an increase in HIV-resistant T-cells and (b) a requirement for much higher numbers of gene-modified cells to achieve therapeutic benefit.

Methods: CCR5-targeting megaTAL is a novel nuclease architecture that combines a LAGLIDADG homing endonuclease scaffold with a 10.5 repeat transcription activator-like (TAL) effector array to achieve efficient site-specific cleavage. The P140K O6-methylguanine-DNA-methyltransferase (MGMT P140K) construct effectively delivers chemo-resistance against the drug O6-benzylguanine/1,3-bis(2-chloroethyl)-1-nitrosourea (BG/BCNU). We are coupling megaTAL nuclease treatment with O6BG/BCNU chemoselection in order to disrupt the CCR5 locus, and positively select modified CD4+ T-cells to achieve therapeutically relevant levels of HIV-protected cells.

Results: Electroporation with megaTAL mRNA demonstrated robust CCR5 disruption: >90% in GHOST cell lines and 80-90% in human CD4+ T-cells. Gene-modified human T cells were transplanted into NOD/SCID/yc-null mice and subsequently challenged with HIV-1 infection. CCR5-null modified cells preferentially survived during active HIV infection in vitro (5 fold increase) and in vivo (100 fold increase). Primary T-cells were transduced with a MGMT P140K chemoselection cassette at 60% efficiency. Preliminary data suggests a BCNU dose-dependent selection and survival of these modified CD4+ T-cells in vitro.

Conclusions: The CCR5-megaTAL nuclease platform produces the highest level of gene-modified CD4+ T-cells reported to date and protects these cells from subsequent HIV infection. Combining this high level disruption with ex-vivo selection of gene modified T cells is a promising approach towards manufacturing a clinical-grade T cell product.
Maximilian Richter, MSc

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In Vivo Transduction of Mobilized Hematopoietic Stem Cells With an Affinity-Enhanced AD5/35 Vector.

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Background: Current protocols for hematopoietic stem cell (HSC) gene therapy involve the collection of HSCs from donors, in vitro culture, transduction with retrovirus vectors, and retransplantation into myelo-conditioned patients. This approach can lead to loss of pluripotency and engraftment capabilities of HSCs. We therefore explored the potential for in vivo transduction and genome editing of HSCs. Previously, we and others reported that human HSCs express high levels of CD46 and can therefore be efficiently transduced by CD46-targeting adenovirus vectors such as Ad5/35 vectors.

Methods: Cells localized in the bone marrow of human CD46 transgenic mice cannot be transduced by intravenously injected Ad5/35 vectors, due to limited accessibility. Mobilization of these bone marrow cells through a combination of G-CSF and CXCR4 antagonist AMD3100 lead to egress of bone marrow cells and a ~100-fold increase in HSCs in the peripheral blood within one hour after AMD3100 injection. At this time, we injected a GFP-expressing, affinity-enhanced Ad5/35 vector (Ad5/35++) and analyzed GFP expression in the bone marrow and the spleen. In addition, transduced cells were subjected to CFU assays to analyze their clonogenic potential.

Results: Upon mobilization GFP expression could be detected in HSCs of the bone marrow. In contrast, nonmobilized animals did not show GFP expression in bone marrow cells. This indicates that mobilization is required for transduction of HSCs and that transduced cells are able to home back to the bone marrow. Furthermore, transduced HSCs isolated from the bone marrow still showed the potential to form colonies in a CFU assay. In the bone marrow 0.01% of cells were transduced LSK cells.

Conclusions: The observed transduction rates are therapeutically relevant for a ZFN-mediated CCR5 knockout, conferring a selective advantage to transduced cells in a HIV setting. We are also testing whether Ad5/35++ vectors can transduce mobilized human CD45+ cells in humanized NOG mice. We will use this model for in vivo transduction with a helper-dependent Ad5/35 vector that expresses a CCR5-specific ZFN, which will allow us to follow genome-edited CD34+ cells long-term.
Pavitra Roychoudhury, PhD  
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Assessing the Efficiency of AAV-Mediated Delivery of DNA Cleavage Enzymes Targeting Latent Viral Infections Using Mathematical Models

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Background: DNA cleavage enzymes have been proposed as a novel curative strategy for persistent viral infections such as HIV. These enzymes are designed to induce targeted mutagenesis in integrated viral genomes in cells that would be otherwise unaffected by traditional antiviral therapies. A number of enzymes have been proposed including zinc finger nucleases, homing endonucleases and TAL effector nucleases, generally delivered as transgenes within vectors that infect the cells of interest. The choice of vector is crucial in determining the success of the proposed therapy and important considerations include delivery and transduction efficiency in target cells, dosing, immunogenicity and toxicity.

Methods: Here we analyze the delivery efficiency of 8 different serotypes of adeno-associated virus (AAV) vectors for delivering cleavage enzymes targeting HIV in SupT1 cells, using flow cytometry data from experiments previously conducted by Pietz et al. Delivery efficiency was assessed by fitting mathematical models to fluorescence distributions measured through the expression of reporter genes at varying multiplicities of infection (MOIs) to flow data.

Results and Conclusion: Our results show that in the absence of dose-dependent toxicity, delivery of vectors follows a sigmoidal dose-response relationship with saturation of gene expression occurring at MOIs that depend on the serotype used. Using the model, we can determine the minimum dose for a given AAV serotype needed to obtain a desired average number of transgenes per cell. Our models provide a method of dose selection that can be applied to wide range of gene therapy applications that rely on both viral and non-viral vectors.
Michael Samuels, PhD
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Quantitative Nucleic Acid Analysis Using RainDrop Single-Molecule PCR: Absolute Counting of DNA and RNA Using Digital PCR, and Targeted Sequencing Sample Preparation from Low-input Samples for Illumina NGS

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Background: Accurate, sensitive, and precise quantification of HIV encoded nucleic acids (both viral RNA and host-integrated DNA) is a requirement for understanding viral biology, dynamics, and clinical metrics enabling efforts towards an HIV cure. Single-molecule PCR based methods provide advantages for both Next Generation Sequencing (NGS) and Digital PCR (dPCR), enabling multiplexed sample prep for targeted NGS sequencing, or separately allowing researchers to add up digital fluorescent counts of single target nucleic acid molecules for absolute sensitive precise quantification.

Methods And Results: The RainDrop dPCR system was used to quantify DNA (using standard qPCR hydrolysis probes) and RNA (using One-Step RT-dPCR standard reagents) sample inputs. Highly sensitive (<10/1,000,000) and precise (<5%CV) multiplex measurements were seen across a wide (6 logs) dynamic range and spectrum of sample inputs, including integrated HIV DNA or viral RNA multiplexed with endogenous and exogenous controls. Additional capabilities for single molecule-formatted sample preparation for targeted NGS were demonstrated.

Conclusion: Multiplexed single molecule ‘digital’ measurements of HIV DNA or RNA by digital PCR showed high sensitivity and precision over a wide dynamic range using a various assays and sample inputs, including plasma HIV gag RNA. In addition, the single-molecule assay format used provides NGS sample prep with a simple workflow and no additional library preparation, yielding sequencing data with high uniformity and coverage.
In Vivo Analyses of Combinatorial Constructs in the Humanized NSG Mouse Model

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Combinatorial antiretroviral therapy (cART) has been very effective in improving the health and prognosis of HIV infected individuals, but it is not curative in adults. Several studies are underway exploring use of genetic approach in treating HIV infection. The ability to engineer body’s own immune cells to fight infection is a promising approach; the transplantation of modified autologous hematopoietic stem cells has potential to support HIV-1 resistant engrafted human immune cells that can suppress viral replication in absence of anti-retroviral therapy. In our present study, we have tested several novel small RNA based anti-HIV constructs including short hairpin RNAs (shRNAs), small interfering RNAs (siRNAs), nucleolar-localizing RNAs that target the HIV genome. Our approach involved evaluation of gene transduced cells with regard to their in vivo engraftment potential and long-term reconstitution, determine lineage specific differentiation into T, B, macrophage and dendritic cell subsets and evaluate stability, expression levels and antiviral efficacies of anti-HIV-1 gene constructs in differentiated cells. Once the individual constructs are screened, promising candidates will be combined into one single lentiviral vector and safety of the combination during human hematopoietic cell development will be assessed. We have utilized NOD/SCID/γc 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 show that X4-tropic virus is capable of infecting 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. We are in the process of analyzing results from preclinical safety evaluation of the individual anti-HIV constructs and their ability to differentiate into various immune subsets. We are also validating the efficacy of humanized NSG mice in supporting the resting CD4+ T cell infection in the various reservoirs following HIV-1 infection and the subsequent suppression of viremia by cART.
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Helper-Dependent Ad5/35 Vectors for ZFN Mediated Gene Editing in Hematopoietic Stem Cells

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A homozygous Δ32 deletion in the CCR5 gene, found in about 1% of Caucasians, confers a natural resistance to HIV-1. Individuals carrying this mutation are healthy, most likely due to the redundant nature of the chemokine system. In a recent pivotal study it was shown that transplantation of hematopoietic stem/progenitor cells (HSCs) from a donor who was homozygous for CCR5 Δ32 in a patient with acute myeloid leukemia and HIV-1 infection resulted in long-term control of HIV. This finding sparked the development of gene therapy approaches to eliminate CCR5 in HIV target cells, including approaches based on CCR5-knockout by zinc finger nucleases (ZFNs). Recent trials involved the ex vivo transduction of patient CD4+ T-cells with a CCR5-ZFN expressing adenovirus Ad5/35 vectors. More recent attempts have focused on CCR5 gene knock-out in hematopoietic stem cells. Because HSCs are a source for all blood cell lineage, CCR5 knock-out would protect not only CD4 cells but also all remaining lymphoid and myeloid cell types that are potential targets for HIV infection. However first-generation Ad5/35 vectors cause cytotoxicity in HSCs due to background viral gene expression. To address this issue we generated helper-dependent, capsid-modified adenovirus (HD-Ad5/35) vectors carrying zinc-finger nuclease (ZFN) targeting CCR5. Moreover using miRNA regulated system we maximized the production of functional HD-Ad5/35-ZFN-miR virus in producer 293-Cre cells. We demonstrated that an HD-AD5/35 vectors expressing a ZFN transduced and conferred efficient knock-out in primitive human HSCs without affecting the viability and differentiation potential of these cells.
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Generation of in vitro Model of HIV Latency for Different HIV-1 U3 Subtypes

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Background: HIV latency is a major obstacle for eradication of the virus. Countless studies have been conducted to explore the mechanisms of establishment and maintenance of HIV-1 latency. However, these studies have mainly focused on HIV-1 subtype B, despite the extensive sequence diversity in the 5’ long terminal repeat (LTR) among different HIV-1 subtypes. HIV-1 gene expression is largely dependent on inducible host transcription factors that interact with numerous cis-regulatory elements within the U3 region of the 5’-LTR. We recently developed LTR-GFP-IRES-Tat (LGIT) latency models for unique U3 subtype isolates in Jurkat and primary CD4+ T cells, and demonstrated that variability of Sp1 and NF-κB binding site in U3 region among subtypes have a major impact on emergence of latency. In addition, these latent subtypes were efficiently reactivated by treatment with the combination of anti-latency drugs. In this study, we established a primary CD4+ T cell latency model using replication-competent HIV-1 NL4-3-based full-length virus (sLTR) containing subtype-specific U3 regions in order to see their expression properties as well as to test for reactivation using various anti-latency drugs.

Methods: Primary naïve CD4+ T cells were activated and infected with the sLTR virus containing various U3 subtype isolates. Aliquots of the culture supernatant were collected, then analyzed using p24 ELISA. Meanwhile, the infected cells were treated with the integrase inhibitor. After 10 days, the cells were activated with CD3/CD28 antibody conjugated beads or anti-latency drugs (e.g. prostratin and SAHA), either individually or in combination. The reactivation was analyzed by intracellular p24 staining, followed by flow cytometry.

Results: Over the time course assay, we observed differences in replication dynamics among U3 subtypes, which suggested that there might be differences in latency establishment as well as susceptibility to anti-latency drugs. Prostratin and SAHA each reactivated latency at modest levels, and the degree of reactivation varied among subtypes. Importantly, the combination of these drugs showed synergistic reactivation.

Conclusions: Using replication-competent virus, we found that different U3 subtypes exhibit different replication rates, which imply contribution of the architecture of U3 regions to latency establishment. Also, as with our previous observation, the combination of prostratin and SAHA synergistically reactivated latency in CD4+ T cells. These results may contribute to developing new approaches for HIV-1 latency reactivation.
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 an 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 and promising pathway toward an HIV cure.

www.defeatHIV.org
Fred Hutchinson Cancer Research Center was established in 1975 and is one of the world’s leading cancer research institutes. Its interdisciplinary teams of scientists conduct research throughout the world to advance the prevention, early detection and treatment of cancer and other diseases. Fred Hutch’s mission is the elimination of cancer and related diseases as causes of human suffering and death. Fred Hutch researchers pioneered bone-marrow transplantation for leukemia and other blood diseases. This research has cured thousands of patients worldwide and has boosted survival rates for certain forms of leukemia from zero to as high as 85 percent. Recognizing that infectious agents contribute to up to a quarter of the world’s cancers, Fred Hutch researchers also study infectious diseases, including HIV- and AIDS-related malignancies. Fred Hutch’s internationally acclaimed scientists include three Nobel Laureates, a MacArthur fellow, seven members of the National Academy of Sciences, five members of the Institute of Medicine, six members of the American Academy of Arts and Sciences, 11 members of the American Association for the Advancement of Science and eight current and former Howard Hughes Medical Institute investigators.

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.

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Cure Scientific Working Group

The focus of the Curative Therapies for HIV (Cure) Scientific Working Group is to accelerate work toward a cure for 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, to develop novel research questions for the study of curative therapies for HIV.

www.depts.washington.edu/cfar
Virology Division

Department of Laboratory Medicine
University of Washington School of Medicine

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.

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

As part of the School of Medicine, educational opportunities are available for undergraduate and graduate students and post-doctoral trainees within the Virology Division. UW Medicine teaching programs are ranked among the best in the country in the 2013 rankings by U.S. News & World Report.

An environment conducive to the performance of high quality research and development is fostered within the Division. The faculty, staff, and trainees are involved in research and development activities that include developing 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 internationally recognized for their clinical and basic science research.

www.depts.washington.edu/labweb/Divisions/Viro
Conference for Cell & Gene Therapy for HIV Cure Dinner and Reception

Please join us on Tuesday, August 26, 2014 for the Conference on Cell and Gene Therapy for HIV Cure Dinner and Reception at Seattle’s iconic Museum of History and Industry on South Lake Union.

The poster session and happy hour will begin at 5:45 pm. with dinner to follow at 7:00 pm.

Museum of History and Industry (MOHAI):
860 Terry Ave N, Seattle, WA 98109
Menu

— POSTER SESSION / HORS D’ŒUVRES —

THAI BEEF SALAD
taro root crisp, daikon, cilantro, vegetable relish

BLACK MISSION FIG
thyme, salted mascarpone, balsamic vinegar

TOSTONES
garlic mojo, guacamole, cotija cheese

BEER and WINE

— DINNER —

SMOKED HANGER TARTINE
wild mushroom polenta cake, green peppercorn aioli

CHICKEN SOUVLAKI
white wine, paprika, onion, garlic, tzatziki

CIPPOLINI ONION TARTLETS
point reyes goat cheese, paprika

TAMALES
roasted zucchini, poblano peppers, green onion, cheese

GREEN SALAD
radish, cherry tomato, cucumber, sherry vinaigrette

BEER and WINE

— DESSERT —

CHOCOLATE TERRINE
coco nib shortbread, raspberry

LEMON CURD TARTLETS
SHARE AN EVENING WITH

NOBEL LAUREATE
FRANÇOISE BARRÉ-SINOUSSI

the SCIENTIST + ACTIVIST who discovered HIV

WEDNESDAY, AUGUST 27, 2014, 7-8:30 PM
PELTON AUDITORIUM
(THOMAS BUILDING ON FRED HUTCH CAMPUS,
1100 FAIRVIEW AVE. N., SEATTLE, WA)

Dr. Barré-Sinoussi will answer your questions
and share her stories about
the HIV research that brought her fame,
the moment that changed her into an activist,
and what more each of us can do
to end the HIV epidemic.

LIGHT REFRESHMENTS
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FOR MORE INFORMATION Call: 206-667-5810
Email: info@defeathiv.org

COMMUNITY ADVISORY BOARD

The mission of the defeat-HIV Community Advisory Board
is to serve as a communication link and mobilize HIV cure
researchers, their institutions and our communities
to work together to cure HIV.

FRED HUTCHINSON CANCER RESEARCH CENTER
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ANGELS IN AMERICA
PART 1: MILLENNIUM APPROACHES  |  PART 2: PERESTROIKA
BY TONY KUSHNER  |  DIRECTED BY ANDREW RUSSELL

INTIMAN THEATRE FESTIVAL
THE CORNISH PLAYHOUSE AT SEATTLE CENTER
AUGUST 12 - SEPTEMBER 21, 2014
INTIMAN.ORG
amfAR, The Foundation for AIDS Research, is one of the world’s leading nonprofit organizations dedicated to the support of AIDS research, HIV prevention, treatment education, and the advocacy of sound AIDS-related public policy. Since 1985, amfAR has invested more than $3888 million in its programs and has awarded more than 3,300 grants to research teams worldwide.

Among many accomplishments, amfAR supported early studies that led to the development of four of the six main classes of anti-HIV drugs that allow people living with HIV/AIDS to live longer, healthier lives, and amfAR pioneered the research that led to the use of antiretroviral drugs to prevent mother-to-child transmission of HIV.

Today amfAR’s research focus is on the search for a cure for HIV/AIDS, primarily through its support of leading scientists working collaboratively within the amfAR Research Consortium on HIV Eradication (ARCHIE).

www.amfar.org.
Bio-Rad Laboratories, Inc. designs, manufactures, and distributes a broad range of innovative tools and services to the life science research and clinical diagnostics markets. Founded in 1952, Bio-Rad has a global team of more than 7,750 employees and serves more than 100,000 research and industry customers worldwide through the company’s global network of operations. Throughout its existence, Bio-Rad has built strong customer relationships that advance scientific research and development efforts and support the introduction of new technology used in the growing fields of genomics, proteomics, drug discovery, food safety, and medical diagnostics.

The Life Science Group develops, manufactures, and markets a wide range products used for research in functional genomics, proteomics, and food safety. The group ranks among the top five life science companies worldwide, and maintains a solid reputation for quality, innovation, and a longstanding focus on the success of its customers. Bio-Rad’s life science technologies include electrophoresis, imaging, multiplex immunoassay, chromatography, microbiology, bioinformatics, protein function analysis, transfection, amplification, real-time and droplet digital PCR.

The Clinical Diagnostics Group develops, manufactures, sells, and supports a large portfolio of products for laboratory diagnostics. Bio-Rad is a leading specialty diagnostics company and its products are recognized as the gold standard for diabetes monitoring and quality control (QC) systems. The company is also well known for its blood virus testing and detection, blood typing, and autoimmune and genetic disorders testing.

www.bio-rad.com
Gilead Sciences, Inc. is a research-based biopharmaceutical company that discovers, develops and commercializes innovative medicines in areas of unmet medical need. With each new discovery and investigational drug candidate, we seek to improve the care of patients living with life-threatening diseases around the world. Gilead’s therapeutic areas of focus include HIV/AIDS, liver diseases, serious respiratory and cardiovascular conditions, cancer and inflammation. Our portfolio of 16 marketed products includes a number of category firsts, including complete treatment regimens for HIV infection available in a once-daily single pill.

www.gilead.com
Sangamo BioSciences, Inc. is a clinical-stage biotechnology company, based in the San Francisco Bay Area, focused on Engineering Genetic CuresTM for monogenic and infectious diseases. Sangamo is deploying its proprietary zinc finger DNA-binding protein (ZFP) technology platform in therapeutic gene regulation and genome editing. The Company has ongoing Phase 2 clinical trials to evaluate the safety and efficacy of a novel ZFP Therapeutic® for the treatment of HIV/AIDS (SB-728-T) and NGF-AAV for Alzheimer’s disease (CERE-110). Sangamo’s other therapeutic programs are focused on monogenic and rare diseases. The Company has formed a strategic collaboration with Shire International GmbH to develop therapeutics for hemophilia A and B, Huntington’s disease, and other monogenic diseases, and with Biogen Idec for hemoglobinopathies, such as sickle cell disease and beta-thalassemia. It has also established strategic partnerships with companies in non-therapeutic applications of its technology, including Dow AgroSciences and Sigma-Aldrich Corporation.

www.sangamo.com
RainDance Technologies is making complex genetics simple. The company’s ultra-sensitive genomic tools enable better research of novel non-invasive Fluid Biopsy™ applications that should result in more accurate, reliable, cost-effective and early detection of cancer, inherited and infectious diseases. Major research institutions and laboratories around the world rely on RainDance systems’ performance. Based in Billerica, Massachusetts, the company supports customers using RainDrop® Digital PCR and ThunderStorm® Targeted Next-Generation Sequencing Systems through its international sales and service operations as well as a global network of distributors and commercial service providers.

www.raindancetech.com

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Conference registration and travel considerations for Community Advisory Board members from the Martin Delaney Collaboratories of defeatHIV, DARE and CARE have been provided by the generous underwriting support from Gilead Sciences and Sangamo BioSciences, Inc.

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David Palm, MSc, CARE CAB
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This conference would not have been possible without the generosity of our sponsors.

Thank you!

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