

18<sup>TH</sup> ANNUAL

# INTERNATIONAL MEETING of the Institute of Human Virology

[www.ihv.org](http://www.ihv.org)

SEPTEMBER 19-22, 2016

at the Four Seasons Hotel  
Baltimore, MD

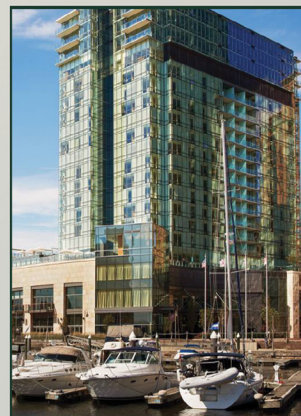
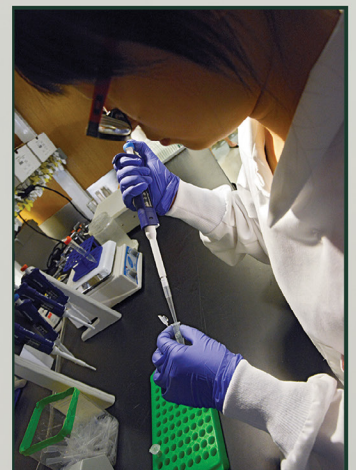


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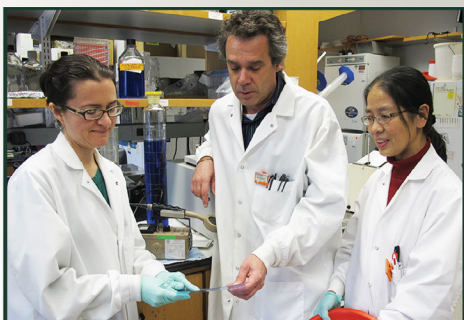
INSTITUTE OF  
HUMAN VIROLOGY  
[www.ihv.org](http://www.ihv.org)



UNIVERSITY of MARYLAND  
SCHOOL OF MEDICINE



Dr. Robert C. Gallo, Director, Institute of Human Virology



Please visit [WWW.IHV.ORG](http://WWW.IHV.ORG) for more information on the program, registration and abstract submission.

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*\*as of press time*

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## Welcome

# 18th Annual International Meeting of the Institute of Human Virology at the University of Maryland School of Medicine

Dear Colleagues and Friends,

The Annual Meeting will feature themes on HIV “Cure” Research with Emphasis on Viral Suppression; Selected New Developments in Cancer Research; Emerging Viruses and the Global Virus Network (GVN); Structural Biology; Extracellular Vesicle Research; Immunology and Viral Pathogenesis Research; Progress in Vaccinology and HIV Prevention; and Advances in Clinical Virology. We have also scheduled a special mini-symposium to honor this year’s **IHV Lifetime Achievement Awardees Raymond Schinazi, PhD, Hon DSc (Public Service) and Peter Vogt, PhD (Scientific Contributions)**. This symposium will feature the third annual Reinhard Kurth Memorial Lecture by Nobel Laureate Harald zur Hausen, among others. To provide opportunities for junior investigators to present, we are emphasizing submission of research abstracts through our web portal - so please share this opportunity with your faculty. As is our tradition, scientific leaders at the cutting-edge of new advances will present Special Lectures on important, emerging topics.

As mentioned, this year’s IHV 2016 Lifetime Achievement Award for Public Service will be presented to Raymond Schinazi, PhD, Hon DSc, Professor of Pediatrics and Director, Laboratory of Biochemical Pharmacology, Emory University and Member, Board of Directors, Global Virus Network (GVN). Dr. Schinazi is being recognized for his outstanding leadership in the field of HIV and his extraordinary ability in translating research to antiviral therapies that have saved the lives of millions of people globally. Harvey Alter, MD, Distinguished National Institutes of Health (NIH) Investigator, Chief, Infectious Diseases Section, Associate Director of Research, Department of Transfusion Medicine, NIH and Mario Stevenson, PhD, Chief, Division of Infectious Diseases, Miller School of Medicine, University of Miami, will lecture in Dr. Schinazi’s honor.

This year’s IHV 2016 Lifetime Achievement Award for Scientific Contributions will be presented to Peter Vogt, PhD, Professor, Department of Molecular and Experimental Medicine, The Scripps Research Institute, California. Dr. Vogt is being recognized for his pioneering role in the study of the genetics, replication cycle and mechanisms of cancer induction by animal retroviruses. Carl Croce, MD, Professor

and Chair, Department of Molecular Virology, Immunology and Medical Genetics, The Ohio State University College of Medicine and Nobel Laureate Harald zur Hausen will present the Reinhard Kurth Memorial Lecture in honor of Dr. Vogt. Dr. Schinazi and Dr. Vogt will be officially honored at our Annual Awards Gala on Wednesday, September 21 at the Four Seasons Hotel in Baltimore. A gala reception will begin at 6:15 pm followed by dinner at 7:00 pm.

We welcome you to Baltimore, Maryland – home of the IHV -- and look forward to you joining us at the elegant Four Seasons Hotel located in Baltimore’s enchanting urban setting and internationally renowned Inner Harbor, which is just a short distance from the National Aquarium the Maryland Science Center.

Meeting participants will find a range of shopping, dining, and cultural venues in this bustling city locale, nicknamed “Charm City” for its historic prominence and delightful red brick row house neighborhoods and sparkling skyscrapers.

We look forward to the IHV 18th Annual International Meeting continuing the tradition of excellent science and provocative discussion.

Sincerely,



**Robert C. Gallo, MD**  
*Homer & Martha Gudelsky Distinguished Professor in Medicine*  
Director,  
*Institute of Human Virology*



**Manhattan Charurat, PhD**  
*Director, Division of Epidemiology and Prevention*  
Associate Professor of Medicine  
*Institute of Human Virology*

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## MISSION STATEMENT

The Institute was established to create and develop a world-class center of excellence focusing on chronic viral diseases, especially HIV/AIDS, and virally-linked cancers.

The IHV is dedicated to the discovery, research, treatment and prevention of these disease.

Its unique structure seeks to connect cohesive, multi-disciplinary research and clinical programs so that new treatments are streamlined from discovery to patient. The IHV serves patients locally and the scientific community globally.

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Associate Director  
Director, Division of Clinical Care and Research

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Director, Division of Animal Models

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The Honorable Ernest F. Hollings  
Ray Lewis  
Princess Chulabhorn Mahidol

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University of Alabama at Birmingham  
Center for AIDS Research

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The Scripps Research Institute

Mario Stevenson, PhD  
University of Miami

Sten Vermund, PhD  
Vanderbilt University

## Organizing Committee

The Institute of Human Virology at the University of Maryland School of Medicine is grateful for the assistance provided by our International and Local Organizing Committees.

Robert C. Gallo, MD, Chair

Carl Dieffenbach, PhD  
 Division of AIDS, National Institute of  
 Allergy and Infectious Diseases

Warner Greene, MD, PhD  
 Gladstone Institute, University of  
 California San Francisco

Jose Esparza, MD, PhD, Co-Chair

Luigi Buonaguro, MD  
 National Cancer Institute, "Fondazione  
 Pascale"

Robert Siliciano, MD, PhD  
 Johns Hopkins University

Anders Vahlne, MD, PhD  
 Karolinska Institutet

John Moore, PhD  
 Weill Cornell Medical College

## Local Organizing Committee

The Institute of Human Virology at the University of Maryland School of Medicine is grateful for the assistance provided by our Local Organizing Committee.

Robert C. Gallo, MD, Chair

Wuyuan Lu, PhD  
 Institute of Human Virology

Man Charurat, PhD, Co-Chair

Fabio Romerio, PhD  
 Institute of Human Virology

Eric Sundberg, PhD  
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 Institute of Human Virology



## Communications and Press Policy

To enhance the exchange of information and communication among attendees of the Institute of Human Virology Annual International Meeting, the following must be adhered to by all participants:

- All comments at sessions are off-the-record and are not for attribution.
- No coverage, reporting or publication of scientific data or presentations at the Institute of Human Virology Annual Meeting is permitted without the written consent of the presenter(s) and Nora Grannell (info below). This rule applies to all forms of media, including blogging, tweeting, etc.
- Alternatively, if the content does not contain information about non-published data, or comments made during the closed meeting, all forms of media are acceptable without written consent.

One-on-one interviews with scientists and media may be arranged by contacting Nora Grannell, Director of Public Relations and Marketing, Institute of Human Virology, (410) 706-1954 or [ngrannell@ihv.umaryland.edu](mailto:ngrannell@ihv.umaryland.edu).

Those registering for the meeting as “press” must provide their credentials within 3 days to [ihvmeeting@ihv.umaryland.edu](mailto:ihvmeeting@ihv.umaryland.edu).



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## Special Acknowledgements

The Institute of Human Virology at the University of Maryland would like to thank the following organizations. Without their continued and generous support, this meeting would not be possible.

National Institute of Allergy and Infectious Diseases\*

Division of AIDS, NIH

Gilead

National Institute on Drug Abuse

AstraZeneca \*

*AZ support is for educational purposes only*

Profectus BioSciences, Inc.

Merck

Abbott Molecular

The Marlene and Stewart Greenebaum Cancer Center

Ortho Clinical Diagnostics

*\*Funding for this conference was made possible [in part] by 2R13AI046078-17 from the National Institute of Allergy and Infectious Diseases. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government.*

## **The 2016 IHV Lifetime Achievement Award for Scientific Contributions and Public Service Peter Vogt, PhD**



Peter Vogt was trained as a virologist at the Max-Planck-Institute in Tübingen, Germany, and at the University of California in Berkeley. His work deals with retroviral replication and genetics, with viral and cellular oncogenes and with the identification of novel inhibitors of oncoproteins. He has made groundbreaking contributions to our knowledge of the cellular and molecular biology and to the genetics of retroviral infections, including the interaction between viral and cellular receptors, genetic recombination between retroviruses, and endogenous retroviral genomes. His discovery of the first temperature-sensitive mutant of Rous sarcoma virus provided definitive proof for the existence of oncogenes.

His work on the structure of retroviral RNA identified a specific sequence responsible for oncogenic transformation, now known as the src oncogene. This work led directly to the discovery of the cellular origin of viral oncogenes. Vogt's studies of diverse retroviruses resulted in the discovery of several novel oncogenes that have become household words in cellular signaling and are of key importance in human cancer: myc, jun and PI 3-kinase. His recent work involves collaborations with chemists at the Scripps Research Institute in a quest for small molecule regulators of cancer targets, notably protein-protein interactions involving the myc protein.

Vogt has held faculty positions at the University of Colorado, the University of Washington, the University of Southern California and is currently Professor at The Scripps Research Institute. Dr. Vogt has received numerous prestigious international honors including the 2010 Albert Szent-Györgyi Prize for Progress in Cancer Research and the 2013 Pezcoller Foundation-AACR International Award for Cancer Research.

## **The 2016 IHV Lifetime Achievement Award for Scientific Contributions and Public Service**

### **Ray Schinazi, PhD, Hon DSc**



Dr. Raymond F. Schinazi is the Frances Winship Walters Professor of Pediatrics and Director of the Laboratory of Biochemical Pharmacology at Emory University and Director of the HIV CURE Working Group for the NIH-sponsored Emory University Center for AIDS Research (CFAR). Dr. Schinazi has authored over 500 peer-reviewed papers and 7 books and holds over 100 issued U.S. patents, which have resulted in 15 New Drug Applications (NDA).

A world leader in nucleoside chemistry, Dr. Schinazi is best known for his pioneering work on HIV, HBV and HCV drugs d4T (stavudine), 3TC (lamivudine), FTC (emtricitabine/Emtriva), LdT (telbivudine), and most recently sofosbuvir (Sovaldi), which are now approved by the FDA. More than 94% of HIV-infected individuals in the US on combination therapy take at least one of the drugs he invented.

Dr. Schinazi served on the Presidential Commission on AIDS and is the recipient of numerous awards including the 2015 William S. Middleton Award from the Department of Veterans Affairs. He is internationally recognized as one of the most influential persons in the life science sector.

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## Previous Recipients of IHV Lifetime Achievement Awards

### LIFETIME ACHIEVEMENT AWARDS FOR SCIENTIFIC CONTRIBUTIONS

- 1999 George Klein, MD, Karolinska Institute, Stockholm, Sweden
- 2000 Maurice Hilleman, PhD, Merck Research Laboratories, Sumneytown, Pennsylvania, USA
- 2001 Hilary Koprowski, MD, Thomas Jefferson University, Philadelphia, Pennsylvania, USA
- 2002 Alexander Rich, MD, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA
- 2003 Jan Svoboda, PhD, DSc, Institute of Molecular Genetics, Prague, Czech Republic
- 2004 Paul Zamecnik, MD, Massachusetts General Hospital, Boston, Massachusetts, USA
- 2005 Manfred Eigen, PhD, Max Planck Institute, Göttingen, Germany
- 2006 Maxine Singer, PhD, National Institutes of Health, Bethesda, Maryland, USA
- 2008 Isaac P. Witz, PhD, Tel Aviv University, Tel Aviv, Israel
- 2010 Rino Rappuoli, PhD, Novartis Vaccines, Siena, Italy
- 2011 Max Essex, DVM, PhD, Harvard AIDS Institute, Boston, Massachusetts, USA
- 2012 Thomas A. Waldmann, MD, National Cancer Institute, Bethesda, Maryland, USA
- 2013 Vadim I. Agol, MD, PhD, DSc, Russian Academy of Medical Sciences, Moscow, Russia
- 2014 William Paul, MD, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

### LIFETIME ACHIEVEMENT AWARD FOR PUBLIC SERVICE

- 2004 Stewart Greenebaum, Greenebaum and Rose Associates, Inc., Baltimore, Maryland, USA
- 2006 Martin Delaney, Project Inform, San Francisco, California, USA
- 2008 John D. Evans, Evans Telecommunication Company, Miami, Florida, USA  
The Honorable Robert K. Gray, Gray and Company II, Miami, Florida, USA
- 2010 Harry Huger, Esq., The Harry and Reba Huger Foundation, Charleston, South Carolina, USA
- 2011 Bernadine Healy, MD, In Memoriam, Former Director National Institutes of Health, Bethesda, MD, USA
- 2012 Yi Zeng, PhD, China Centers for Disease Control, Beijing, China
- 2013 José G. Esparza, MD, PhD, Bill & Melinda Gates Foundation, Seattle, Washington, USA
- 2014 John Martin, PhD, Gilead Sciences, Inc., Foster City, California, USA

### ONE-TIME LIFETIME ACHIEVEMENT AWARD FOR EXCELLENCE IN TEACHING

- 2010 Michele LaPlaca, MD, Institute of Microbiology of the University of Bologna, Bologna, Italy

### LIFETIME ACHIEVEMENT AWARD FOR EXCELLENCE IN MEDICAL EDUCATION, CLINICAL CARE AND CLINICAL RESEARCH

- 2012 John G. Bartlett, MD, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

### LIFETIME ACHIEVEMENT AWARD FOR SCIENTIFIC CONTRIBUTIONS AND PUBLIC SERVICE

- 2015 Harald zur Hausen, MD, Nobel Laureate, Gelsenkirchen, Germany
- 2015 Anthony S. Fauci, MD, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA



## Evening Events Schedule

**Monday, September 19, 2016**  
6:30 – 8:15 pm

**Opening Reception**  
*Grand Prefunction*

**Tuesday, September 20, 2016**  
6:30 – 8:15 pm

**Poster Session**  
*Grand Prefunction*

**Wednesday, September 21, 2016**  
6:30 pm

**Lifetime Achievement Awards  
Gala Reception**  
*Grand Prefunction*

7:15 pm

**Lifetime Achievement Gala Banquet  
and Awards Ceremony**  
*Cobalt*

## Program Overview

### Monday, September 19, 2016

8:20 am – 12:20 pm

**Session A - HIV “Cure” Research with Emphasis on Viral Suppression**

10:00 – 10:20 am

**Coffee Break**

12:20 – 1:35 pm

**Lunch**

1:35 – 3:15 pm

**Session B - Selected New Developments in Cancer Research**

3:15 – 3:35 pm

**Coffee Break**

3:35 – 5:50 pm

**Session C - Emerging Viruses and the Global Virus Network**

6:30 – 8:15 pm

**Opening Reception**

### Tuesday, September 20, 2016

8:20 am – 12:40 pm

**Session D - Structural Biology**

10:00 – 10:20 am

**Coffee Break**

12:20 – 1:35 pm

**Lunch**

1:35 – 3:15 pm

**Session E - Extracellular Vesicle Research**

3:15 – 3:35 pm

**Coffee Break**

3:35 – 5:45 pm

**Session F - Immunology and Viral Pathogenesis Research**

6:30 – 8:15 pm

**Poster Session**

**Program Overview, cont.**

**Wednesday, September 21, 2016**

8:20 am – 12:35 pm

**Session G - Progress in Vaccinology and HIV Prevention**

10:40 – 10:55 am

**Coffee Break**

12:45 – 1:50 pm

**Lunch**

1:50 – 5:40 pm

**Session H - Lifetime Achievement Award Mini-Symposium**

4:00 – 4:20 pm

**Coffee Break**

6:30 pm

**Gala Reception**

7:15 pm

**Lifetime Achievement Awards Dinner**

**Thursday, September 22, 2016**

8:20 am – 12:20 pm

**Session I - Advances in Clinical Virology**

10:10 – 10:30 am

**Coffee Break**

11:50 am

**Closing Remarks**

## Speaker Schedule

**Monday, September 19, 2016**

**Session A:**

**HIV “Cure” Research with Emphasis on Viral Suppression**

**Grand Ballroom**

Chairpersons and Discussants:

**Carl Dieffenbach, PhD**, National Institute of Allergy and Infectious Diseases

**Anders Vahlne, MD, PhD**, Karolinska Institutet

- 8:20    Robert Siliciano, MD, PhD, Johns Hopkins University  
*Defective proviruses rapidly accumulate during acute HIV-1 infection* **A-101**
- 8:40    Bruce Walker, MD, Ragon Institute of MGH, MIT, and Harvard  
*Impact of treatment in Fiebig Stage I on HIV-specific immune responses: Implications for cure strategies* **A-102**
- 9:00    Guido Poli, MD, Vita-Salute San Raffaele University  
*Towards Achieving a State of Reversible HIV-1 Latency in Primary Monocyte-Derived Macrophages (MDM) by M1 Polarization* **A-103**
- 9:20    Jonathan Karn, PhD, Case Western Reserve University  
*Distinct mechanisms of hormonal control of HIV latency in T-cells and microglial cells* **A-104**
- 9:40    J. Victor Garcia-Martinez, PhD, University of North Carolina  
*In vivo analysis of the myeloid HIV reservoir in the CNS* **A-105**

**Coffee Break, 10:00 AM - 10:20 AM** Grand Prefunction

- 10:20    Ashley Haase, MD, University of Minnesota  
*Concentrating Antibodies at Mucosal Frontlines for Prevention* **A-106**
- 10:40    Timothy Schacker, MD, University of Minnesota  
*How Important Is the Lymphoid Tissue Reservoir?* **A-107**
- 11:00    Steven Wolinsky, MD, Northwestern University  
*Persistent viral replication maintains the tissue reservoir during drug therapy* **A-108**
- 11:20    Fabio Romerio, PhD, Institute of Human Virology  
*The HIV-1 antisense transcript AST promotes latency by recruiting PRC2 to the 5’LTR* **A-109**
- 11:40    Session Speakers, co-chaired by Carl Dieffenbach, PhD and Robert Gallo, MD  
*Special Panel Discussion on HIV Cure Research* **A-110**

**Lunch, 12:20 PM – 1:35 PM**



**Session B:**

**Selected New Developments in Cancer Research**

**Grand Ballroom**

Chairpersons and Discussants:

**Eduardo Sotomayor, MD**, George Washington University School of Medicine & Health Sciences

**Franco Buonaguro, MD**, Istituto Nazionale Tumori “Fondazione Pascale”

- |      |   |              |
|------|---|--------------|
| 1:35 | Riccardo Dalla-Favera, MD, Columbia University<br><i>Molecular Genetics of HIV-associated B-cell Lymphomas</i>  | <b>B-101</b> |
| 1:55 | Gary Borisy, PhD, The Forsyth Institute<br><i>Visualizing the Complexity of Microbiomes at the Micron Scale</i>   | <b>B-102</b> |
| 2:15 | Robert Burk, MD, Albert Einstein College of Medicine<br><i>Sexual transmission of HPV16 from Neandertals to modern humans and the evolution of viral oncogenesis</i>  | <b>B-103</b> |
| 2:35 | Bernhard Fleckenstein, MD, Universitätsklinikum Erlangen<br><i>Functional Dissection of Primary Immunodeficiencies by Rhadinovirus-Mediated T-Cell Transformation</i> | <b>B-104</b> |
| 2:55 | Jeffrey Schlom, PhD, National Cancer Institute<br><i>Emerging Concepts in Cancer Immunotherapy</i>  | <b>B-105</b> |

**Coffee Break, 3:15 PM - 3:35 PM** Grand Prefunction

**Session C:**

**Emerging Viruses and the Global Virus Network**

**Grand Ballroom**

Chairpersons and Discussants:

**Kathleen Neuzil, MD, MPH**, Institute of Global Health, University of Maryland School of Medicine

**Manhattan Charurat, PhD**, Institute of Human Virology

- |      |   |              |
|------|---|--------------|
| 3:35 | Jerome Kim, MD, International Vaccine Institute<br><i>The Middle East Respiratory Syndrome (MERS) experience in Korea</i>   | <b>C-101</b> |
| 3:55 | Scott Weaver, PhD, Institute for Human Infections and Immunity, University of Texas Medical Branch<br><i>Zika Virus: History, Evolution, Transmission, Emergence Mechanisms, and Activities of the GVN Task Force</i> | <b>C-102</b> |
| 4:15 | Alan Schmaljohn, PhD, University of Maryland School of Medicine<br><i>Special Lecture: Beyond Neutralization is Metaneutralization: Precedents and Complexities with Emerging Viruses</i>                             | <b>C-103</b> |
| 4:40 | Roger Glass, MD, PhD, Fogarty International Center, National Institutes of Health<br><i>Special Lecture: Rotavirus and Rotavirus Vaccines: Current status and future challenges</i>                                   | <b>C-104</b> |
| 5:05 | Konstantin Chumakov, PhD, U.S. Food and Drug Administration<br><i>A new generation of poliovirus vaccines and antiviral tools</i>   | <b>C-105</b> |

- 5:25 A.D.M.E. (Ab) Osterhaus, PhD, DVM, University of Veterinary Medicine Hannover, Germany  
*Special Lecture: Emerging infections in animals and humans* **C-106**
- 5:50 Diane Griffin, MD, PhD, Johns Hopkins Bloomberg School of Public Health  
*Special Lecture: Measles, a re-emerging disease* **C-107**

**Opening Reception 6:30 PM - 8:15 PM** Grand Prefunction

## Tuesday, September 20, 2016

### Session D:

#### Structural Biology

##### Grand Ballroom

Chairpersons and Discussants:

**Eric Sundberg, PhD**, Institute of Human Virology

**Leonid Margolis, PhD**, National Institute of Child Health and Human Development

- 8:20 Stefan Sarafianos, PhD, University of Missouri School of Medicine  
*Structural Basis of Inhibition and Resistance Mechanism to EFdA, a highly potent NRTI* **D-101**
- 8:40 Andrew Ward, PhD, The Scripps Research Institute  
*The Dynamic HIV-1 Envelope Glycoprotein Trimer* **D-102**
- 9:00 Sriram Subramaniam, PhD, National Cancer Institute  
*Cryo-EM of dynamic molecular assemblies* **D-104**
- 9:20 Peijun Zhang, PhD, The Scripps Research Institute  
*Structural Basis of HIV-1 Capsid Assembly, Maturation and Host Cell Interactions* **D-105**
- 9:40 Marzena Pazgier, PhD, Institute of Human Virology  
*Structural targeting of the A32-region epitopes for antibody-dependent cell-mediated cytotoxicity* **D-106**

**Coffee Break, 10:00 AM - 10:20 AM** Grand Prefunction

- 10:20 Jason McLellan, PhD, Dartmouth College  
*Structure and Stabilization of Coronavirus Spike Proteins in the Prefusion Conformation* **D-107**
- 10:40 Bing Chen, PhD, Harvard University  
*Structural Basis for Membrane Anchoring of HIV-1 Envelope Spike* **D-108**
- 11:00 Joseph Sodroski, MD, Harvard University  
*Understanding and Exploiting the Conformational States of the HIV-1 Envelope Glycoprotein Trimer* **D-109**
- 11:20 Gregory Melikian, PhD, Emory University School of Medicine  
*Real-time imaging of single HIV-1 core uncoating* **D-110**

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- 11:40 Carol Weiss, MD, PhD, U.S. Food and Drug Administration  
*Coordinated gp41 and gp120 mutations conferring an open conformation of Env and their consequences on Env function* **D-111**
- 12:00 Pamela Bjorkman, PhD, California Institute of Technology  
*Structure of a natively-glycosylated HIV-1 Env reveals a new mode for VH1-2 antibody recognition of the CD4 binding site relevant to vaccine* **D-112**

**Lunch, 12:20 PM - 1:35 PM**

**Session E:**

**Extracellular Vesicle Research**

**Grand Ballroom**

Chairpersons and Discussants:

**Thomas Lehner, MD**, King's College London

**Isaac Witz, PhD**, Tel Aviv University

- 1:35 Leonid Margolis, PhD, National Institute of Child Health and Development  
*Extracellular vesicles released by HIV-1 infected cells carry viral proteins and facilitate HIV infection of human lymphoid tissue* **E-101**
- 1:55 Fatah Kashanchi, PhD, George Mason University  
*Exosomes from retrovirus infected cells carry distinct viral noncoding RNAs and proteins that control the fate of the recipient cell* **E-102**
- 2:15 Dirk Dittmer, PhD, University of North Carolina School of Medicine  
*Viral exosomes exert paracrine effects on endothelial cells leading to enhanced migration* **E-103**
- 2:35 Howard Fox, MD, PhD, University of Nebraska Medical Center  
*Extracellular vesicle microRNA leads to neurotoxicity in SIV infection* **E-104**
- 2:55 Esther N.M. Nolte-t Hoen, PhD, Utrecht University  
*Naked virions, extracellular vesicles, and vesicle-enclosed virions released early after picornavirus infection - who, when, how, and why?* **E-105**

**Coffee Break, 3:15 PM - 3:35 PM** Grand Prefunction

**Session F:**

**Immunology and Viral Pathogenesis Research**

**Grand Ballroom**

Chairpersons and Discussants:

**Guido Poli, MD**, Vita-Salute San Raffaele University

**Arnaldo Caruso, MD**, University of Brescia Medical School

- 3:35 Louis Picker, MD, Oregon Health & Science University  
*Special Lecture: Programming CD8+ T Cell Immunity with Cytomegalovirus Vectors* **F-101**
- 4:00 Warner Greene, MD, PhD, Gladstone Institute of Virology and Immunology  
*Special Lecture: On Death and Dying with HIV: Pyroptosis Drives CD4 T Cell Depletion* **F-102**

- 4:25 Paolo Lusso, MD, PhD, National Institute of Allergy and Infectious Diseases  
*Structure-Function Elucidation of the Native HIV-1 Envelope Trimer As a Basis for Rational Vaccine Design* **F-103**
- 4:45 Jeffrey Lifson, MD, National Cancer Institute  
*Insights Into AIDS Virus Pathogenesis from Studies in Nonhuman Primate Models* **F-104**
- 5:05 Timothy Fouts, PhD, Profectus Biosciences  
*Vesiculovirus vectored vaccines can provide single dose protection against filoviruses, arenaviruses, and alphaviruses* **F-105**
- 5:25 John Mellors, MD, University of Pittsburgh  
*Clonally-Amplified Proviruses as Reservoirs of HIV* **F-106**

**Poster Session 6:30 PM - 8:15 PM** Grand Prefunction

## Wednesday, September 21, 2016

### Session G:

#### Progress in Vaccinology and HIV Prevention

##### Grand Ballroom

Chairpersons and Discussants:

**Robert C. Gallo, MD, Director**, Institute of Human Virology  
**Georgia Tomaras, PhD**, Duke Human Vaccine Institute

- 8:20 Donald Forthal, MD, University of California, Irvine  
*Non-neutralizing antibody activities: the good, bad and indifferent* **G-101**
- 8:40 Margaret Ackerman, PhD, Dartmouth College  
*Fine epitope signature of HIV-1 antibody neutralization breadth at the CD4 binding site* **G-102**
- 9:00 Gabriel Victora, PhD, Whitehead Institute for Biomedical Research  
*Clonal and cellular dynamics in antibody evolution* **G-103**
- 9:20 Thomas Hope, PhD, Northwestern University Feinberg School of Medicine  
*Defining the earliest targets of SIV susceptibility after mucosal challenge in the Rhesus Macaque model* **G-104**
- 9:40 Anthony DeVico, PhD, Institute of Human Virology  
*HIV Vaccines Based on Transition State Envelope Structures* **G-105**
- 10:00 Christopher Parks, PhD, International AIDS Vaccine Initiative  
*Mucosal vaccination with a replication-competent VSV-HIV chimera delivering Env trimers protects rhesus macaques from rectal SHIV infection* **G-106**
- 10:20 Garnett Kelsoe, DSc, Duke University School of Medicine  
*High Resolution of Humoral Responses to HIV-1: determinism or chance?* **G-107**

**Coffee Break, 10:40 AM - 10:55 AM** Grand Prefunction



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- 10:55 Michel Nussenzweig, MD, PhD, The Rockefeller University  
*Special Lecture: HIV-1 Prevention: Progress Towards Passive or Active Vaccination* **G-108**
- 11:20 Jeffrey Ravetch, MD, PhD, The Rockefeller University  
*Special Lecture: Engineering anti-HIV antibodies for optimal control of HIV infection* **G-109**
- 11:45 Cynthia Derdeyn, PhD, Emory University School of Medicine  
*Events in Early HIV-1 Infection That Prime the Development of Heterologous Neutralization Breadth* **G-110**
- 12:05 Frances Eun-Hyung Lee, MD, Emory University School of Medicine  
*Identification of Human Long-lived Plasma Cells: Implications for HIV Vaccines* **G-111**
- 12:25 Thomas Lehner, MD, King's College London  
*The effect of stress agents in vitro and human vaccination in vivo on stem cell memory CD4+ CD45- T cells* **G-112**

**Lunch, 12:45 PM - 1:50 PM**

**Session H:**

**Lifetime Achievement Award Mini-Symposium**

**Grand Ballroom**

Chairpersons and Discussants:

**Robert C. Gallo, MD**, Director, Institute of Human Virology

**William Blattner, MD**, IHV Co-founder

- 1:50 Robert C. Gallo, MD, Director, Institute of Human Virology  
*Introduction to Lifetime Achievement Awards* **H-101**
- Carlo Croce, MD, The Ohio State University College of Medicine  
*Video remarks in honor of Peter Vogt*
- 1:55 Robin Weiss, MD, PhD, University College London  
*Speaking in honor of Peter Vogt: Pseudoviruses: Sheep in Wolves' Clothing* **H-102**
- 2:15 Joseph Pagano, MD, University of North Carolina  
*Speaking in honor of Peter Vogt: The Epstein-Barr Virus, a 50-year Odyssey* **H-103**
- 2:35 Harald zur Hausen, MD, Nobel Laureate, German Cancer Research Center  
*Special Lecture in honor of Peter Vogt: Novel infectious agents in dairy cattle and their role in human chronic diseases* **H-104**
- 3:15 Peter Vogt, PhD, The Scripps Research Institute, The 2016 IHV Lifetime Achievement Award for Scientific Contributions  
*Reinhard Kurth Memorial Lecture: The Non-coding Transcriptome: Regulation by MYC and Cancer-specific Transcripts* **H-105**

**Coffee Break, 4:00 PM - 4:20 PM** Grand Prefunction

- 4:20 Mario Stevenson, PhD, University of Miami Miller School of Medicine  
*Speaking in honor of Ray Shinazi: Assessing the contribution of myeloid cells to HIV-1 persistence in the face of ART* **H-106**
- 4:40 Samuel Broder, MD, Intrexon  
*Speaking in honor of Ray Shinazi: Thirty Years of Anti-Retroviral Therapy for Patients with AIDS* **H-107**
- 5:00 Harvey Alter, MD, National Institutes of Health  
*Special Lecture in honor of Ray Schinazi: The HCV Story: From Origins to Cure* **H-108**
- 6:30 PM Gala Reception** Grand Prefunction
- 7:15 PM Lifetime Achievement Awards Dinner** Cobalt

## Thursday, September 22, 2016

### Session I:

#### Advances in Clinical Virology

##### Grand Ballroom

Chairpersons and Discussants:

**Shyamasundaran Kottilil, MD, PhD**, Institute of Human Virology

**John Bartlett, MD**, Johns Hopkins University School of Medicine

- 8:20 John Bartlett, MD, Johns Hopkins University School of Medicine  
*Public Health Approach to HIV Stagnation* **I-101**
- 8:40 Mark Wainberg, MD, McGill University  
*The Absence of Drug Resistance against Dolutegravir in First-Line Therapy is Attributable to Reduced Viral Replicative Fitness* **I-103**
- 9:00 Barry Peters, MD, King's College London  
*Metabolic and cardiovascular co-morbidities in people living with HIV* **I-104**
- 9:20 Mark Sulkowski, MD, Johns Hopkins University School of Medicine  
*Advances in the treatment of chronic HCV infection with direct acting antivirals* **I-105**
- 9:40 Howard Gendelman, MD, University of Nebraska  
*Special Lecture: Transforming anti-HIV drugs* **I-106**

**Coffee Break, 10:10 AM - 10:30 AM** Grand Prefunction

##### Grand Ballroom

Chairpersons and Discussants:

**Ed Tramont, MD**, National Institute of Allergy and Infectious Diseases

**Robert Redfield, MD**, Institute of Human Virology

- 10:30 Shyamasundaran Kottilil, MD, PhD, Institute of Human Virology  
*Challenges in eradicating chronic HBV infection* **I-107**

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- 10:50 Luigi Buonaguro, MD, National Cancer Institute “Fondazione Pascale”  
*Discovery to first-in-man of a multi-peptide-based hepatocellular carcinoma vaccine adjuvanted with CV8102 (RNAJuvant) - HEPAVAC* **I-108**
- 11:10 Peter Stock, MD, University of California, San Francisco  
*Transplantation in the HIV Positive Recipient: The Unexpected Findings* **I-109**
- 11:30 JoAnn Suzich, PhD, MedImmune  
*Targeting the PD-1/PD-L1 pathway to achieve a functional cure for chronic infection* **I-110**
- 11:50 Robert Gallo, MD, Institute of Human Virology  
*Closing Remarks* **I-111**

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Robert Siliciano, MD, PhD, Johns Hopkins University
- A-102 *Impact of treatment in Fiebig Stage I on HIV-specific immune responses: Implications for cure strategies*  
Bruce Walker, MD, Ragon Institute of MGH, MIT, and Harvard
- A-103 *Towards Achieving a State of Reversible HIV-1 Latency in Primary Monocyte-Derived Macrophages (MDM) by M1 Polarization*  
Guido Poli, MD, Vita-Salute San Raffaele University
- A-104 *Distinct mechanisms of hormonal control of HIV latency in T-cells and microglial cells*  
Jonathan Karn, PhD, Case Western Reserve University
- A-105 *In vivo analysis of the myeloid HIV reservoir in the CNS*  
J. Victor Garcia-Martinez, PhD, University of North Carolina
- A-106 *Concentrating Antibodies at Mucosal Frontlines for Prevention*  
Ashley Haase, MD, University of Minnesota
- A-107 *How Important Is the Lymphoid Tissue Reservoir?*  
Timothy Schacker, MD, University of Minnesota
- A-108 *Persistent viral replication maintains the tissue reservoir during drug therapy*  
Steven Wolinsky, MD, Northwestern University
- A-109 *The HIV-1 antisense transcript AST promotes latency by recruiting PRC2 to the 5'LTR*  
Fabio Romerio, PhD, Institute of Human Virology
- A-110 *Special Panel Discussion on HIV Cure Research*  
Session Speakers, co-chaired by Carl Duffenbach and Robert Gallo
- B-101 *Molecular Genetics of HIV-associated B-cell Lymphomas*  
Riccardo Dalla-Favera, MD, Columbia University
- B-102 *Visualizing the Complexity of Microbiomes at the Micron Scale*  
Gary Borisy, PhD, The Forsyth Institute
- B-103 *Sexual transmission of HPV16 from Neandertals to modern humans and the evolution of viral oncogenesis*  
Robert Burk, MD, Albert Einstein College of Medicine
- B-104 *Functional Dissection of Primary Immunodeficiencies by Rhadinovirus-Mediated T-Cell Transformation*  
Bernhard Fleckenstein, MD, Universitätsklinikum Erlangen
- B-105 *Emerging Concepts in Cancer Immunotherapy*  
Jeffrey Schlom, PhD, National Cancer Institute
- C-101 *The Middle East Respiratory Syndrome (MERS) experience in Korea*  
Jerome Kim, MD, International Vaccine Institute



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Scott Weaver, PhD, Institute for Human Infections and Immunity, University of Texas Medical Branch
- C-103 *Special Lecture: Beyond Neutralization is Metaneutralization: Precedents and Complexities with Emerging Viruses*  
Alan Schmaljohn, PhD, University of Maryland School of Medicine
- C-104 *Special Lecture: Rotavirus and Rotavirus Vaccines: Current status and future challenges*  
Roger Glass, MD, PhD, Fogarty International Center, National Institutes of Health
- C-105 *A new generation of poliovirus vaccines and antiviral tools*  
Konstantin Chumakov, PhD, U.S. Food and Drug Administration
- C-106 *Special Lecture: Emerging infections in animals and humans*  
A.D.M.E. (Ab) Osterhaus, PhD, DVM, University of Veterinary Medicine Hannover, Germany
- C-107 *Special Lecture: Measles, a re-emerging disease*  
Diane Griffin, MD, PhD, Johns Hopkins Bloomberg School of Public Health
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Stefan Sarafianos, PhD, University of Missouri School of Medicine
- D-102 *The Dynamic HIV-1 Envelope Glycoprotein Trimer*  
Andrew Ward, PhD, The Scripps Research Institute
- D-104 *Cryo-EM of dynamic molecular assemblies*  
Sriram Subramaniam, PhD, National Cancer Institute
- D-105 *Structural Basis of HIV-1 Capsid Assembly, Maturation and Host Cell Interactions*  
Peijun Zhang, PhD, The Scripps Research Institute
- D-106 *Structural targeting of the A32-region epitopes for antibody-dependent cell-mediated cytotoxicity*  
Marzena Pazgier, PhD, Institute of Human Virology
- D-107 *Structure and Stabilization of Coronavirus Spike Proteins in the Prefusion Conformation*  
Jason McLellan, PhD, Dartmouth College
- D-108 *Structural Basis for Membrane Anchoring of HIV-1 Envelope Spike*  
Bing Chen, PhD, Harvard University
- D-109 *Understanding and Exploiting the Conformational States of the HIV-1 Envelope Glycoprotein Trimer*  
Joseph Sodroski, MD, Harvard University
- D-110 *Real-time imaging of single HIV-1 core uncoating*  
Gregory Melikian, PhD, Emory University School of Medicine
- D-111 *Coordinated gp41 and gp120 mutations conferring an open conformation of Env and their consequences on Env function*  
Carol Weiss, MD, PhD, U.S. Food and Drug Administration

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- E-101 *Extracellular vesicles released by HIV-1 infected cells carry viral proteins and facilitate HIV infection of human lymphoid tissue*  
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- E-102 *Exosomes from retrovirus infected cells carry distinct viral noncoding RNAs and proteins that control the fate of the recipient cell*  
Fatah Kashanchi, PhD, George Mason University
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Dirk Dittmer, PhD, University of North Carolina School of Medicine
- E-104 *Extracellular vesicle microRNA leads to neurotoxicity in SIV infection*  
Howard Fox, MD, PhD, University of Nebraska Medical Center
- E-105 *Naked virions, extracellular vesicles, and vesicle-enclosed virions released early after picornavirus infection - who, when, how, and why?*  
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Louis Picker, MD, Oregon Health & Science University
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Warner Greene, MD, PhD, Gladstone Institute of Virology and Immunology
- F-103 *Structure-Function Elucidation of the Native HIV-1 Envelope Trimer As a Basis for Rational Vaccine Design*  
Paolo Lusso, MD, PhD, National Institute of Allergy and Infectious Diseases
- F-104 *Insights Into AIDS Virus Pathogenesis from Studies in Nonhuman Primate Models*  
Jeffrey Lifson, MD, National Cancer Institute
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Timothy Fouts, PhD, Profectus Biosciences
- F-106 *Clonally-Amplified Proviruses as Reservoirs of HIV*  
John Mellors, MD, University of Pittsburgh
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Donald Forthal, MD, University of California, Irvine
- G-102 *Fine epitope signature of HIV-1 antibody neutralization breadth at the CD4 binding site*  
Margaret Ackerman, PhD, Dartmouth College
- G-103 *Clonal and cellular dynamics in antibody evolution*  
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- H-104 *Special Lecture in honor of Peter Vogt: Novel infectious agents in dairy cattle and their role in human chronic diseases*  
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- H-105 *Reinhard Kurth Memorial Lecture: The Non-coding Transcriptome: Regulation by MYC and Cancer-specific Transcripts*  
Peter Vogt, PhD, The Scripps Research Institute, The 2016 IHV Lifetime Achievement Award for Scientific Contributions

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Michael Odutola, MD, Institute of Human Virology, Nigeria
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Yutaka Tagaya, MD, PhD, Institute of Human Virology
- P-A3 *Expression of Evolutionarily Novel Genes in Tumors*  
Ekaterina A Matyunina, Junior Researcher, The Biomedical Center and Peter the Great St. Petersburg Polytechnic University
- P-A4 *Distinct subcellular localization of HTLV-1 HBZ oncoprotein in the ATL leukemic and HAM/TSP patients*  
Roberto S. Accolla, MD, PhD, University of Insubria
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Franco M Buonaguro, MD, Istituto Nazionale Tumori - IRCCS "Fondazione Pascale", Napoli, Italy
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Virginia Carroll, PhD, Institute of Human Virology, University of Maryland School of Medicine
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Mikalus Popovic, MD, PhD, Institute of Human Virology
- P-A8 *Therapeutic targeting of intracellular toll-like and interleukin-1 receptor signaling for inhibiting infection, inflammation, and cancer.*  
Greg Snyder, PhD, Institute of Human Virology, Department of Medicine, School of Medicine, University of Maryland, Baltimore, MD.
- P-A9 *Molecular Studies in the HIV-1 Transgenic mouse with PCNS lymphoma*  
Tapas K. Makar, PhD, Institute of Human Virology
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Ursula Dietrich, Dr., Georg-Speyer-Haus, Frankfurt, Germany
- P-B2 *HIV Envelope gp120 fused to IgG1 Fc fragment exhibits augmented immunogenicity compared to unmodified immunogen and elicits a neutralizing antibody response in rhesus macaques*  
Zhanna Shubin, PhD Candidate, Ihv
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Glenn CL Wong, University of Oxford
- P-C2 *Characterization of the Early Antibody Landscape In HIV-1 Infected Individuals Who Develop Poor to Elite Levels of Neutralization Breadth*  
S. Abigail Smith, PhD, Emory University
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Juan C Zapata, PhD, Institute of Human Virology, University of Maryland School of Medicine
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Qingbo Liu, PhD, NIAID, NIH
- P-C7 *Differential host gene expression in HIV-1 and HIV-2 infected monocyte derived macrophages (MDM)*  
Santanu Biswas, PhD, LMV/DETTD/OBRR/CBER/FDA, Silver Spring, MD 20993
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Eleanor Wilson, MD, MHS, Institute of Human Virology, University of Maryland School of Medicine
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- P-C10 *HIV, Hepatitis C, Hepatitis B Co- Infections And Their Genotypes Among Fishermen Attending Homabay District Hospital*  
Allan O. Onyango, MS, NIH
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Genoveffa Franchini, MD, NIH
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Lydia Tang, MBChB, Institute of Human Virology, University of Maryland School of Medicine
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Joseph Bryant, DVM
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Preetha Balasubramanian, MS, NYU school of medicine
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Christina Guzzo, PhD, Laboratory of Immunoregulation, NIAID



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James Arthos, PhD, Niaid
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Alfredo Garzino-Demo, PhD, Institute of Human Virology, and Dept. of Microbiology and Immunology, Dept. of Molecular Medicine, University of Padova, Italy
- P-C21 *Molecular Mimicry of a Helical Region in Domain 1 of CD4 Facilitates Interdomain Stabilization of HIV-1 gp120*  
Peng Zhang, PhD, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA
- P-C22 *The constitutive activation of NF- $\kappa$ B by HTLV-1 Tax-1 oncoprotein is inhibited by the MHC class II transactivator CIITA*  
Roberto S Accolla, MD, PhD, University of Insubria
- P-C23 *Spleen tyrosine kinase (Syk) controls IFN- $\alpha$  production inhibited in plasmacytoid dendritic cells by surface glycoproteins of HIV, HBV and HCV*  
Ivan Hirsch, PhD, Centre de Recherche en Cancérologie de Marseille, Inserm U1068, Marseille, France, CNRS, UMR7258, Marseille, France, Institut Paoli-Calmettes, Marseille, France, Aix-Marseille Université, UM105, Marseille, France, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Department of Genetics and Microbiology, Faculty of Science, Charles University in Prague, Prague, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic
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A-101

*Defective proviruses rapidly accumulate during acute HIV-1 infection***Robert Siliciano, MD, PhD**, Johns Hopkins University

Although antiretroviral therapy (ART) suppresses viral replication to clinically undetectable levels, HIV-1 persists in CD4+ T cells in a latent form not targeted by the immune system or ART1-5. This latent reservoir is a major barrier to cure. Many patients initiate ART during chronic infection, and in this setting, most proviruses are defective<sup>6</sup>. However, the dynamics of the accumulation and persistence of defective proviruses during acute HIV infection are largely unknown. Here we show that defective proviruses accumulate rapidly within the first few weeks of infection to make up over 93% of all proviruses in treated patients, regardless of how early ART is initiated. Using an unbiased screening method to amplify near full-length proviral genomes from HIV-1 infected adults treated at different stages of infection, we demonstrate that early ART initiation limits the size of the reservoir but does not profoundly impact its composition. This analysis allows us to revise our understanding of the composition of the reservoir and estimate the true reservoir size in individuals treated early vs. late in infection. Additionally, we demonstrate that commonly used assays for measuring the reservoir do not correlate with reservoir size. These findings reveal hurdles that must be overcome to successfully analyze future HIV-1 cure strategies.

A-102

*Impact of treatment in Fiebig Stage I on HIV-specific immune responses: Implications for cure strategies***Bruce Walker, MD**, Ragon Institute of MGH, MIT, and Harvard

HIV incidence rates approach 10% per year in young African women in KwaZulu Natal Province, South Africa. In this study we linked a pathway out of poverty program to a surveillance program for acute HIV infection. Termed FRESH, for Females Rising through Education, Support and Health, the study engages women aged 18-23 in Umlazi Township in a twice-weekly curriculum of empowerment and life skillstraining, HIV prevention education, and job readiness, with the goal of having each participant become employed or return to school at the end of one year. At each twice weekly visit, a finger prick blood draw is performed for HIV RNA detection. At first detection of viral RNA, combination antiviral therapy (cART) is initiated. Of the 29 cases of treated acute infection, approximately 75% have been in Fiebig Stage I. The lowest peak viremia achieved with immediate cART was 440 RNA copies/ml plasma, with viremia cleared in less than 7 days. The impact of limited antigen exposure on the development of humoral and cellular immune responses will be discussed.



A-103

*Towards Achieving a State of Reversible HIV-1 Latency in Primary Monocyte-Derived Macrophages (MDM) by M1 Polarization***Guido Poli, MD,** Vita-Salute San Raffaele University

Background - We have previously reported that short-term exposure of primary MDM to pro-inflammatory cytokines (IFN- $\gamma$  plus TNF- $\alpha$ ), i.e. "M1 polarization", partially prevented productive virus infection and reduced proviral transcription. Materials & Methods -M1-polarized MDM were restimulated with M1 cytokines 7 days after R5 HIV-1 infection (M1x2 protocol). Cell cultures were monitored for supernatant-associated RT activity, HIV-1 DNA load, APOBEC3G/3A expression, and for cell reactivation by coculture with T cell blasts. Results - We observed a significant, further reduction of virus replication down to near undetectable levels by RT activity over 30 days of culture. HIV-1 DNA levels were ca. 100- and 1,000-fold lower in M1-MDM and M12-MDM vs. control, unpolarized cells, respectively. APOBEC3A, but not APOBEC3G, was significantly upregulated by the M12 protocol protocol 15 days post-infection. No effect of T cell blast coculture on control, infected MDM was observed, whereas significant levels of RT activity were induced in M12-MDM by this approach. Discussion - Stimulation of already infected M1-MDM with pro-inflammatory, M1-cytokines counter-intuitively resulted in a further, significant inhibition of virus replication down to "near-latency" levels in terms of RT activity and viral DNA levels and upregulation of APOBEC3A expression. Recovery of virus production was achieved by cocultivation of M12-infected MDM with allogeneic T cell blasts, indicating the existence of a pool of infected cells carrying inducible proviruses.

A-104

*Distinct mechanisms of hormonal control of HIV latency in T-cells and microglial cells***Jonathan Karn, PhD,** Case Western Reserve University

Kien Nguyen, Hong-Xia Mao, Biswajit Das, Curtis Dobrowolski &amp; Jonathan Karn

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Histone lysine methyltransferases (HKMTs) are key mediators of epigenetic silencing. Our previous work demonstrated a critical role for the H3K27MT EZH2, the catalytic subunit of the Polycomb repressive complex 2 (PRC2), for the maintenance of HIV-1. In this study, we showed that depletion of individual subunits of PRC2 by shRNA partially reactivated and sensitized latent HIV-1 proviruses to exogenous stimulations in latently infected Jurkat T-cell lines. Chromatin immunoprecipitation (ChIP) experiments demonstrated that PRC2 and G9a were highly enriched at proviral 5' LTR and rapidly displaced upon TNF- $\alpha$  reactivation. Depletion of EZH2 expression by shRNA in Jurkat T cells or inhibition of EZH2 methyltransferase activity in primary T cells before HIV-1 infection significantly reduced levels of silent viruses. Similarly, shRNA depletion of G9a or inhibition of its enzymatic activity by UNC0638 prior to HIV-1 infections also resulted in decreasing of silent viruses. In addition, inhibition of EZH2 enzymatic activity by GSK-343 or EPZ-6438 in resting memory T cells cultured ex vivo or isolated from HIV-1 infected patients receiving highly active antiviral therapy (HAART) caused spontaneous reactivation of latent proviruses. Treatment of resting memory T cells isolated from HIV-1 infected patients with UNC-0638 also partially reactivated latent proviruses, even though shRNA depletion of G9a had relatively little impact on reactivation of latent proviruses in Jurkat T cells. We conclude that both PRC2 and G9a are required for the establishment and maintenance of HIV-1 proviral silencing in primary cells. Furthermore, HK3MT inhibitors, such as GSK-343, EPZ-6438 or UNC-0638, are highly potent as latency-reversing agents (LRAs) when combined with other agents to reactivate latent HIV-1 ex vivo.

A-105

*In vivo analysis of the myeloid HIV reservoir in the CNS*

**J. Victor Garcia-Martinez, PhD**, University of North Carolina

HIV infection results in a lifelong condition that when treated resembles a chronic disease. However, despite of years of fully suppressive therapy HIV persists in the host and is never eradicated. Key to HIV persistence is the infection of several different types of cells each of which has been postulated to be an important determinant of pathogenesis and persistence. Macrophages have been long considered to be key contributors to HIV infection and its dissemination into the CNS, a sanctuary somewhat independent of the periphery. However, recent studies have contradicted these early work and suggest that macrophages are not in vivo targets of HIV infection. To address this question we first determined the presence of HIV in monocytes isolated from viremic patients and from patients undergoing antiretroviral treatment. Consistent with these recent reports we failed to find viral DNA in blood monocytes from infected patients. Since the analysis of tissue macrophages is limited by the difficulties associated with invasive procedures we developed and implemented a novel humanized mouse model in which only myeloid cells are susceptible to HIV-1 infection. Using these myeloid only mice we demonstrate 1) that macrophages can sustain HIV replication in vivo in the absence of T cells, 2) that HIV infected macrophages are systemically distributed in all tissues analyzed including the brain, 3) that replication competent virus can be rescued from infected macrophages ex-vivo and 4) that macrophages can establish de novo infection in uninfected animals. These results demonstrate that macrophages represent a bone fide target for HIV infection in vivo that can sustain and transmit infection.

A-106

*Concentrating Antibodies at Mucosal Frontlines for Prevention*

**Ashley Haase, MD**, University of Minnesota

Understanding the robust protection conferred by the live attenuated SIV vaccine, SIV-delta nef, could provide guiding principles for development of an effective HIV vaccine. In studies in the SIV-rhesus macaque model of HIV transmission to women, SIV-delta nef vaccination has been shown to elicit non-neutralizing antibodies that react with trimeric gp41 (gp41t) and are concentrated by the neonatal Fc receptor (FcRn) in cervical-vaginal epithelium on the path of virus entry, as one potential correlate of protection. I will review the pathogenesis studies that revealed this potential correlate and describe the current status of reproducing SIV-delta nef protection with gp41t immunogens, adjuvants and an immunization protocol that mimics the persistent antigen exposure of infection.



A-107

*How Important Is the Lymphoid Tissue Reservoir?*

**Timothy Schacker, MD**, University of Minnesota

Progress towards a cure for HIV infection is hampered by limited understanding of the size, location, and dynamic nature of virus reservoirs, especially the latent reservoir. We have used several in situ hybridization and immunohistochemistry techniques to directly assess the location and frequency of SIV and HIV RNA and DNA positive cells. Our previous studies document the size of these reservoirs, especially in lymphatic tissues however those data were collected in an era when ART was less effective than current regimens. In this talk I will review these data and provide new information on the size and location of virus reservoirs in the modern ART era using modern in situ technologies. I will discuss data from non-human primate studies where more complete tissue analysis can be accomplished from every organ system as well as longitudinal data from HIV infected people before and during ART. These data are especially relevant as we contemplate various strategies to purge these reservoirs in an effort to cure the infection.

A-108

*Persistent viral replication maintains the tissue reservoir during drug therapy*

**Steven Wolinsky, MD**, Northwestern University

Combinations of antiretroviral drugs usually suppress viral replication and reduce viral RNA to undetectable levels in the bloodstream in HIV-1 infection. It is unclear, however, whether treatment fully suppresses viral replication in lymphoid tissue, a key reservoir established by HIV-1 during acute infection. We examined viral sequences in lymphoid tissue and blood from three HIV-1-infected individuals receiving antiretroviral drug therapy. Our approach for the characterization of the viral populations was robust with respect to experimental error and stochastic effect. The evolutionary patterns and population-dynamic processes from the time-structured sequence data show a strong clock-like signal and rates consistent with HIV-1 within-host evolution, suggesting that the antiretroviral drug concentration in the lymphoid tissue is insufficient to completely block virus replication. A mathematical model explains why drug resistance does not necessarily arise under conditions where drug concentrations are insufficient to fully block virus replication. These data reveal the evolutionary and infection dynamics of the virus population within the host, indicating that HIV-1 can continue to replicate and replenish the viral reservoir despite antiretroviral drug therapy.

A-109

*The HIV-1 antisense transcript AST promotes latency by recruiting PRC2 to the 5'LTR*

**Fabio Romerio, PhD**, Institute of Human Virology

Although the HIV-1 Tat protein is necessary for viral exit from latency, the prevailing view has been that HIV-1 does not encode for an inducer of latency, and that environmental stimuli indirectly control entry into latency. Histone methyltransferases (HMTs) contribute to the establishment of viral latency through precise positioning of the nucleosomes Nuc-0 and Nuc-1 on the 5'LTR. The HMT, EZH2 – a component of the Polycomb Repressor Complex 2 (PRC2) – plays the dominant role in this process. Two very important and possibly related questions are still open. First, how are Nuc-0 and Nuc-1 precisely and invariably positioned at the 5'LTR irrespective of the site and orientation of HIV-1 integration into the host genome? Second, long non-coding RNAs (lncRNAs) are recognized as key participants in this process by tethering PRC2 to the chromatin. What is the lncRNA that recruits PRC2 to the HIV-1 5'LTR? An attractive hypothesis that would answer both questions is that HIV-1 has evolved the ability to encode for its own lncRNA as an autonomous mechanism to recruit PRC2 to the 5'LTR, and to establish latency regardless of the chromatin context, integration site and orientation into the host genome. We demonstrate that an antisense transcript (AST) encoded in the HIV-1 genome and directed from an antisense promoter within the 3'LTR suppresses HIV-1 expression by recruiting PRC2 to the 5'LTR, and promoting epigenetic modifications that lead to the establishment and maintenance of viral latency. These results suggest that HIV-1 encodes for an lncRNA that acts an inducer of viral latency. In addition, they could guide in designing new therapies aimed at reversing or stabilizing latency by interfering or exploiting AST.

A-110

*Special Panel Discussion on HIV Cure Research*

Session Speakers, co-chaired by **Carl Dieffenbach, PhD** and **Robert Gallo, MD**



B-101

*Molecular Genetics of HIV-associated B-cell Lymphomas***Riccardo Dalla-Favera, MD**, Columbia University

Molecular Genetics of HIV-associated B-cell Lymphomas Riccardo Dalla-Favera, MD Institute for Cancer Genetics Columbia University, New York NY, 10032 Despite the immune reconstitution promoted by combined antiretroviral therapy (cART), lymphomas still represent the most common type of cancer in HIV- infected individuals. The most common lymphomas arising in HIV-infected individuals include Burkitt lymphoma (HIV-BL) and diffuse large B-cell lymphoma (HIV-DLBCL). Analogous to their counterparts occurring in immune-competent individuals, these malignancies derive from mature B cells involved in the germinal centers (GC), the site where antigen-stimulated B cells undergo selection for antigen affinity. The pathogenesis of these lymphomas is associated with genetic lesions, and preliminary studies based on limited cytogenetic and targeted gene analysis, have suggested some analogies with the genomic alterations (translocations involving the MYC and BCL6 oncogenes, and p53 inactivation) recurrently detected in non HIV-associated cases. While a comprehensive definition of the coding genome of both HIV-associated lymphomas is still lacking, whole-exome sequencing and copy number variation analysis has allowed the identification of a large number of recurrent genetic lesions in BL and DLBCL. These lesions have in turn identified recurrently altered cellular pathways that point to potential new therapeutic targets. These results will be reviewed as a guide to future comparative studies in HIV-associated lymphomas.

B-102

*Visualizing the Complexity of Microbiomes at the Micron Scale***Gary Borisy, PhD**, The Forsyth Institute

The spatial organization of complex natural microbiomes is critical to understanding the physiology and ecology of microbial communities as well as their interaction with their host and their impact on both health and disease. Although next-generation DNA sequencing technology and metagenomics have revolutionized the analysis of microbial communities, a major gap in our understanding is the lack of spatial information at the micron level. Using spectral imaging fluorescence in situ hybridization as guided by tagged sequence analysis, we have discovered distinctive, multi-genus consortia in the human oral microbiome. The spatial structure of the consortia reveals unanticipated interactions and provides a framework for understanding the organization, metabolism, and systems biology of the microbiome, and ultimately its effect on the health of the human host. Our synthesis of high-throughput sequencing data with spatial and structural information demonstrates the informative value of microbial biogeography at the micron scale.



B-103

*Sexual transmission of HPV16 from Neandertals to modern humans and the evolution of viral oncogenesis***Robert Burk, MD**, Albert Einstein College of Medicine

Human papillomavirus (HPV) type 16, a small circular double-stranded DNA virus, is the most oncogenic HPV and causes more than half of all cervix cancers. HPV16 is also the most common sexually transmitted viral infection. We have characterized and sequenced the complete genomes from HPV16 isolates throughout the world and will discuss viral variant lineages and phylogeny. Although analyses of HPV16 variants originally suggested codivergence with human population movement, recent discoveries on the origin of modern humans from multiple archaic lineages (e.g., Neandertals) suggests a more complex scenario. We have re-evaluated the origins of HPV16, using phylogeny-based approaches and estimated the timing of HPV16 diversion using Bayesian Markov Chain Monte Carlo (MCMC) methods. The divergence time of HPV16 A-lineage (Eurasian) and B/C/D-lineage (African) variants from their most recent common ancestor was estimated at approximately 500,000 years ago, roughly coinciding with the split of modern humans and Neandertals. Moreover, we used principle coordinate analysis (PCoA) to examine the geographic clustering of over 3,000 HPV16 variants. These analyses indicated that the A-lineage variants were significantly under represented in Africa. Taken together, these data suggest that the currently circulating HPV16 A-lineage variants are the result of recent viral sexual transmission from Neanderthals to modern humans. These observations are consistent with multiple genetic introgressions of archaic hominins and modern humans through interbreeding in the past 80,000 years and the massive recent expansion of Eurasian humans. The host genome was also found to influence the pathogenesis of HPV16 variant lineages. The evolution and pathogenesis of HPV16 provide insights into viral-host interactions.

B-104

*Functional Dissection of Primary Immunodeficiencies by Rhadinovirus-Mediated T-Cell Transformation***Bernhard Fleckenstein, MD**, Universitätsklinikum Erlangen

Primary immunodeficiency diseases (PID) are a group of more than 250 relatively rare inherited chronic disorders in which the immune system is improperly functioning, affecting one or more of its components. About one in 500 children or young adults in the United States are born with a primary immunodeficiency, mostly manifesting by recurrent viral, bacterial, fungal, or protozoal infections, severe or long lasting warts, generalized mollusca contagiosa, complications of vaccination, prolonged or recurrent diarrhoea, developmental abnormalities, autoimmunity and malignant tumors such as Kaposi's sarcoma and lymphoproliferative syndromes. A major limitation for the genetics of PID lies in the limited availability of immune cells for a molecular analysis and functional studies. We have developed protocols for the growth transformation of human T-cells in culture by a recombinant rhadinovirus based on Herpesvirus (H.) saimiri, subgroup C strain 488. This allowed for the reproducible generation of T-cell lines from more than 30 distinct clinical situations where mutations could be identified in the genes for membrane-bound ligands, cytokines and cytokine receptors, membrane-bound receptors, non-receptor protein kinases and phosphatase, adapter proteins in signaling, trafficking proteins, fusion accessory proteins, a metabolic enzyme, and transcription factors. These T-cell lines have proven useful for structural and functional analyses in diseases such as autoimmune lymphoproliferative syndrome (ALPS), Hyper-IgE-syndrome (HIES), chronic mycobacteriosis, childhood-onset Kaposi's sarcoma, epidermodysplasia verruciformis (EV), and Epstein Barr virus-associated lymphoproliferation and lymphoma.





B-105

*Emerging Concepts in Cancer Immunotherapy***Jeffrey Schlom, PhD**, National Cancer Institute

Immune-mediated immunotherapeutics as single agents are now being employed as the standard-of-care in several cancer types. However, for the vast majority of patients with solid tumors, immunotherapy has yet to have a major impact. Numerous modes of immunotherapy are currently being evaluated in our program at the National Cancer Institute, NIH, in both preclinical and clinical studies. These include (a) three diverse recombinant vaccine platforms targeting tumor-associated antigens and a transcription factor (brachyury) that has been shown to drive "stemness" and epithelial to mesenchymal transition (EMT); (b) checkpoint inhibitor monoclonal antibodies such as anti-PDL1 and anti-CTLA4; (c) immune modulators such as anti-IL8, and a tumor-targeting IL-12 immunocytokine, and the IL15/Ra/Fc immunocytokine; and (d) an allogeneic "off the shelf" NK cell, which contains high granzyme levels and expresses a high avidity CD16 for enhanced antibody-dependent cell-mediated cytotoxicity (ADCC). Recent preclinical and clinical studies have also shown that so-called "non-immune-based" standard-of-care therapies can alter the phenotype of tumor cells to render them more susceptible to immune-mediated attack, and/or enhance the balance of effector to immune regulatory cells. A thorough interrogation of both the tumor and tumor microenvironment, and the peripheral immunome, will also be needed to define which patients are most likely to benefit from immunotherapy protocols. Different immunotherapeutics have been shown to (a) activate different arms of the immune system, (b) enhance ongoing immune responses, and/or (c) remove immune inhibitory entities. The challenge now exists as to how to employ combinations of these diverse immunotherapeutics with or without so-called "non-immune-based" therapies for optimal patient benefit.

C-101

*The Middle East Respiratory Syndrome (MERS) experience in Korea***Jerome Kim, MD** International Vaccine Institute

Jerome H. Kim, MD; Manki Song, PhD; In-kyu Yoon, MD

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In 2015 Korea experienced the largest outbreak of MERS outside of Saudi Arabia. Despite its impressive development and health care system, there were some features of the epidemic that were specific to Korea and others that were more typical of outbreaks elsewhere. Idiopathic features included Korean health seeking behavior, medical service in facilities, and visits to sick friends/family. Further analysis of molecular sequences suggests that there were genetic differences and a key question is whether these difference account for clinical features seen during the outbreak. Some evidence of a superspreader phenotype has been found. During the time since the outbreak a number of groups have initiated work on vaccines, and a DNA vaccine from GeneOne has been tested in humans. Within Korea, questions around the use of DNA vaccines delayed consideration of these candidates until legal issues were clarified and the first candidate under development is a DNA vaccine. A second, protein nanoparticle approach is also being developed. In all these trials aim to have candidates through Phase II testing for future potential development.

C-102

*Zika Virus: History, Evolution, Transmission, Emergence Mechanisms, and Activities of the GVN Task Force*

**Scott Weaver, PhD**, Institute for Human Infections and Immunity, University of Texas Medical Branch

Zika virus (ZIKV), a mosquito-borne flavivirus, was first isolated from a sentinel rhesus macaque in Uganda and soon thereafter from arboreal *Aedes africanus* mosquitoes. Although only 14 human infections were documented before 2007, human serosurveys in African and Asia suggested widespread exposure. Following 60 years of relative obscurity, ZIKV emerged in Yap, a small Micronesian Island, to cause just over 100 confirmed and suspected cases of febrile illness accompanied by rash and arthralgia, and epidemiologic studies suggested that up to 73% of the human population was infected. A few years, larger outbreaks began in French Polynesia and other islands in the South Pacific, with an estimated tens-of-thousands of infections and an association of some with Guillain–Barré syndrome (GBS), whose incidence increased 20-fold coincident with the ZIKV outbreak. Then, probably in late 2013, ZIKAV reached Brazil, resulting in 2015 in an explosive outbreak involving over one million estimated cases, presumably transmitted by the peridomestic mosquito *A. aegypti* and possibly also the invasive species *A. albopictus*. Sexual transmission has also been detected in travelers returning to non-endemic regions. In the Americas, Zika virus infections were again associated with GBS but also with over 1,400 confirmed cases of fetal microcephaly coincident in time and space with ZIKV circulation. Subsequently, the virus has spread to the majority of countries in Latin America and the Caribbean, and microcephaly has also appeared in several of these. Here, I review the current situation of ZIKV in the Americas, potential explanations for its sudden and unexpected emergence, and epidemiologic and basic research needed to test these hypotheses to understand these the dramatic spread, predict future trends, and develop control measures as well as products to protect against severe outcomes of infection. I will also review efforts of the GVN Zika Task force to facilitate collaborative research and educate the public about this emerging virus.

C-103

*Special Lecture: Beyond Neutralization is Metaneutralization: Precedents and Complexities with Emerging Viruses*

**Alan Schmaljohn, PhD**, University of Maryland School of Medicine

Emerging viruses of high if intermittent concern for human health (exemplified here by filoviruses, alphaviruses, flaviviruses, and poxviruses) tend to cause acute and—unlike HIV—relatively non-persistent infections. While complete prevention of infection by vaccines is goalworthy, mitigation of viral burden is generally sufficient. Antibodies play a prominent role in both short-term and lasting immunity; however, these viruses also demonstrate how the complexities of antibody-mediated resistance to viral infection, disease, and spread are under-served by the common language of virology, in which assay-based terms such as neutralization and ADCC (antibody-dependent cell-mediated cytotoxicity) promote unhelpful oversimplifications of the in vivo phenomena they are intended to describe. For instance, it is widely inferred in error that something called neutralization is reliably necessary and sufficient for antibody-mediated protection against viruses. The roles and targets of antibodies against Ebola/Marburg, Sindbis/chikungunya, dengue/Zika and vaccinia/monkeypox viruses serve to contradict simple views. To provoke and expand new language among experts for whom nuance and complexity matter greatly (especially those involved in research on and development of vaccines and antibody-based therapies), the term metaneutralization is used here to encompass all mechanisms (entry-level neutralization, ADCC, and other) resident in antiviral protection observed empirically when monoclonal or polyclonal antibodies are administered before or after viral infection of an animal. For all mechanisms, appropriate Fc type and optimal Fc-FcR interactions appear helpful—often decisive—in favorable outcomes.

C-104

*Special Lecture: Rotavirus and Rotavirus Vaccines: Current status and future challenges*

**Roger Glass, MD, PhD**, Fogarty International Center, National Institutes of Health

Rotavirus vaccines have been introduced into the routine immunization programs of more than 80 countries. Their impact has been rapid with the reduction in diarrheal illnesses, hospitalizations, and deaths. With this introduction have been a number of changes in the epidemiology of rotavirus infections that could not have been properly anticipated beforehand including the demonstration of herd or community protection in non-vaccinated children, older children and adults, the changing age distribution of disease, a decrease in seizure related hospitalizations, and what may be a new biannual distribution of disease peaks similar to what was seen for measles before routine vaccination. Unfortunately, these live oral vaccines have proven to be less effective in some low income settings where they are needed most. Research has identified a number of possible explanations – interference from simultaneous OPV administration, high titers of transplacental antibodies, and differences in the microbiome of children in these settings, none of which are easy to change. Consequently, we have embarked on the development of a parenteral inactivated rotavirus vaccine (IRV) with the hope that this product would improve the efficacy of a rotavirus vaccine in all settings while avoiding recurrent concerns about intussusception that has occurred with all these live oral rotavirus vaccines. Immunization of piglets with an IRV has stimulated cross-reacting neutralization antibodies while protecting these animals against virus shedding when challenged with wild type rotavirus strains. Work is ongoing to develop a candidate vaccine suitable for testing in humans.

C-105

*A new generation of poliovirus vaccines and antiviral tools*

**Konstantin Chumakov, PhD**, U.S. Food and Drug Administration

Inactivated and Live (Oral) vaccines against poliomyelitis are among the most successful vaccines ever. They have brought about virtual elimination of the disease, and it is expected that transmission of all wild polioviruses will stop within one or two years. Even though poliovirus may soon be eradicated, polio immunization cannot stop because gaps in population immunity would create a risk of re-starting poliovirus circulation leading to potentially catastrophic consequences. On the other hand the existing vaccines cannot be used in post-eradication period. Reversion of strains used in oral polio vaccine (OPV) creates pathogenic vaccine-derived polioviruses causing outbreaks of the disease. Inactivated poliovirus vaccine (IPV) is expensive, does not prevent virus spread, and is manufactured from virulent strains, causing biosecurity concerns. Efforts are underway to create a new generation of polio vaccines for post-eradication use and antiviral tools to complement vaccines. IPV could be made from safer strains, e.g. from attenuated Sabin strains. This product, however, does not completely address manufacturing safety concerns. Engineered strains with higher stability were proposed, as well as production schemes that do not require live virus. New strains were also proposed for a new generation of improved OPV, which is currently in pre-clinical development. Finally, drugs and monoclonal antibodies active against poliovirus could be used to treat chronically infected individuals and for emergency response. All these efforts can succeed only through effective public-private partnership, and this presentation will focus on regulatory research needed to enable timely introduction these new products.



C-106

*Special Lecture: Emerging infections in animals and humans*

**A.D.M.E. (Ab) Osterhaus, PhD, DVM**, University of Veterinary Medicine Hannover, Germany  
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Complex relationships between human and animal species have resulted in human-animal interfaces that have promoted cross-species transmission, emergence and eventual evolution of a plethora of human pathogens. Remarkably, most of the characteristics of these interfaces have been established long before the end of our species pre-historical development, to be relentlessly shaped throughout the history of our own and animal species. More recently, changes affecting the modern human population worldwide and their dramatic impact on the global environment have taken domestication, agriculture, urbanization, industrialization, and colonization to unprecedented levels. This has created new global multi-faceted human-animal interfaces, associated with major epidemiological transitions, accompanied by an unexpected rise of emerging and re-emerging infectious diseases in humans, that all have their origin in animal reservoirs. Until the beginning of the last century, infectious diseases were the major cause of mortality of humans. Around 1900 infectious diseases caused about fifty percent of human deaths in the western world. In the following decades, this percentage decreased to less than a few percent. This was largely due to the implementation of public health measures such as sewage installment and development of clean drinking water systems, but also to development of vaccines and antimicrobials. Major successes in this regard were the eradication of smallpox and rinderpest through well-orchestrated vaccination campaigns in humans and cattle, respectively. Such successes prompted policymakers and scientists to predict that infectious diseases of humankind and of their domestic animals would eventually be brought under control in the industrialized world. Paradoxically the following decades confronted the world with an ever-increasing number of emerging or re-emerging infectious diseases, some causing true human or animal pandemics. Pathogens spilling over from wildlife reservoirs, either directly or via intermediate hosts, were at the basis of most of these disease outbreaks. Striking examples in humans were the emergence of AIDS from chimpanzees, avian flu from migratory birds, and SARS, MERS, and Ebola from bat reservoirs. A complex mix of predisposing factors in our globalizing world, linked to major changes in our societal environment and global ecology, collectively created opportunities for viruses and other pathogens to infect and adapt to new animal and/or human hosts. This paved the way for the unprecedented spread of infections in humans and animals with dramatic consequences for public and animal health, animal welfare, food supply, economies, and biodiversity. It is important to realize that due to the complex and largely interactive nature of the predisposing factors, it is virtually impossible to predict what the next pathogen threat will be, from where it will come and when it will strike. However better understanding of the underlying processes may eventually lead to predictions that would improve our preparedness for outbreaks in humans and animals. Importantly, the increased emergence of viral infections is largely paralleled by medical, veterinary, technological, and scientific progress, continuously spurred by our never-ending combat against pathogens. Investment in better understanding the human-animal interfaces will therefore offer a future head start in the never-ending battle against infectious diseases of humans.

C-107

*Special Lecture: Measles, a re-emerging disease*

**Diane Griffin, MD, PhD**, Johns Hopkins Bloomberg School of Public Health Baltimore, MD

Measles virus (MeV) is a highly infectious respiratory virus that causes a systemic rash disease associated with substantial morbidity and mortality. Infection results in a viremia followed by persistence of viral RNA in blood and lymphoid tissue for many months, immune suppression and increased susceptibility to other infections. An efficacious and safe live attenuated virus vaccine is in widespread use. Because of the need for high population immunity to prevent endemic transmission, 2 doses are required. Delivery of the second dose in resource poor parts of the world has relied on mass campaigns that have been difficult to sustain and measles has returned in many of these countries. Because of philosophical objections to vaccination, outbreaks of measles have also been increasing in industrialized nations that once had eliminated endemic transmission.



D-101

*Structural Basis of Inhibition and Resistance Mechanism to EFdA, a highly potent NRTI*

**Stefan Sarafianos, PhD**, University of Missouri School of Medicine

4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) is the most potent nucleoside analog inhibitor of HIV reverse transcriptase (RT). It is stable and well tolerated in clinical trials (MK-8591), having the potential for once-weekly oral dosing (Hazuda, CROI 2016). EFdA retains a 3'-OH, yet acts as a chain-terminator by diminishing translocation from the pre-translocation Nucleotide-binding site (N-site) to the post-translocation Primer-binding site (P-site). To understand the high potency and unusual inhibition mechanism of EFdA we solved crystal structures representing intermediates of EFdA blocking DNA synthesis. Two of these show EFdA bound at the N- and P-sites, providing molecular insights of EFdA inhibition as an immediate or delayed chain terminator. The structures show that EFdA blocks RT translocation through favorable interactions of its 4'-ethynyl (4'-E) at a conserved hydrophobic pocket at the N-site, and unfavorable interactions of 4'-E at the P-site, leading to localized primer distortion. Passages of HIV in presence of a 4'-E NRTI selected RT resistance mutations. The primary mutation, M184V, conferred 7-fold resistance to EFdA. Secondary substitutions T165R and I142V increased resistance to 22-fold. Importantly, the mutations reduced replication capacity. To identify the resistance mechanism we solved structures of M184V/T165R/I142V, M184V/T165R, and M184V in complex with DNA and EFdA-triphosphate (TP). Replacement of the flexible Met side-chain with a rigid b-branched Val in M184V, mildly decreased EFdA-TP binding, leading to resistance through steric hindrance. T165R in  $\alpha$ -helix E blocked contacts of R165 with Q182, enabling interactions with 184 of the YMDD loop, which in turn binds incoming dNTP and EFdA-TP. Hence, M184V and T165R confer EFdA resistance by decreasing its incorporation. In contrast, I142V helps unblock EFdA-terminated primers. Hence, unlike with other NRTIs, multiple resistance mechanisms are needed for only a 22-fold resistance to EFdA, making it a promising antiviral.

D-102

*The Dynamic HIV-1 Envelope Glycoprotein Trimer*

**Andrew Ward, PhD**, The Scripps Research Institute

There are now several high-resolution structures of HIV-1 envelope glycoprotein (Env) trimer, the key target for broadly neutralizing antibodies. Nearly all structures however are of the soluble, stabilized, prefusion conformation of Env, and there is little variability between the structures. It is well appreciated that Env is a flexible, meta-stable protein capable of undergoing large receptor-induced conformational changes to perform its role in host cell recognition and entry. Here we use cryo-electron microscopy (cryoEM) to study several different conformations of Env, including the CD4 bound state, at high-resolution to describe the molecular details that stabilize these intermediate states and enable Env to function. Additionally, we compare the structures of the soluble stabilized trimers to those of the wild-type transmembrane containing Env to derive insights for HIV vaccine development.



D-104

*Cryo-EM of dynamic molecular assemblies***Sriram Subramaniam, PhD**, National Cancer Institute

Recent breakthroughs in the field of cryo-electron microscopy provide new prospects for determination of the structures of a variety of macromolecular assemblies and small dynamic protein complexes. The prospect that the determination of protein structures to atomic resolution will no longer be limited by size, or by the need for crystallization represents a significant and exciting horizon in structural biology. I will discuss the application of these methods to analyze structures of a variety of biologically and medically relevant multi-protein complexes and membrane protein assemblies, which have historically represented the most challenging frontier in structural biology. Selected publications: Merk A., Bartesaghi A, Banerjee S, Falconieri V, Rao P, Davis M, Pragani R, Boxer M, Earl LA, Milne JLS, Subramaniam S (2016) Breaking cryo-EM resolution barriers to facilitate drug discovery. *Cell*, 165 1698-1707. Matthies D, Dalmás, O, Borgnia, MJ, Dominik, PK, Merk, A, Rao, P, Reddy, BG, Islam, S., Bartesaghi, A, Perozo, E, Subramaniam, S (2016) Cryo-EM Structures of the Magnesium Channel CorA Reveal Symmetry Break Upon Gating. *Cell*, 164, 747-756. Banerjee, S, Bartesaghi, A, Merk, A, Rao, P, Bulfer, SL, Yan, Y, Green, N, Mroczkowski, B, Neitz, RJ, Wipf, P, Falconieri, V, Deshaies, RJ, Milne, JLS, Huryn D, Arkin, M, Subramaniam S (2016) 2.3 Å resolution cryo-EM structure of human p97 and mechanism of allosteric inhibition. *Science*, 351,871-875. Narayan K, Subramaniam, S (2015) Focused ion beams in biology. *Nat Methods*, 12:1021-1031. Bartesaghi A, Merk A, Banerjee S, Matthies D, Wu X, Milne JLS, Subramaniam S (2015) 2.2 Å resolution cryo-EM structure of  $\beta$ -galactosidase in complex with a cell-permeant inhibitor. *Science* 348:1147-1151.

D-105

*Structural Basis of HIV-1 Capsid Assembly, Maturation and Host Cell Interactions***Peijun Zhang, PhD**, The Scripps Research Institute

HIV-1 capsid plays critical roles in HIV-1 replication by protecting the genome from innate immune sensing response and interacting with many host factors including CypA, CPSF6, MxB, TRIM5 $\alpha$  and TRIM-Cyp. We have previously determined the CA tubular assembly to 8 Å using cryoEM and built an all-atom computer model of the complete capsid by large scale molecular dynamics (MD) simulations. Exploiting the recent advance in direct electron detection, we have now obtained the structure of HIV-1 capsid at near-atomic resolution, revealing functionally important elements in an assembly context. Our novel in vitro HIV-1 maturation process and computer simulations further suggest a probable assembly pathway for HIV-1 maturation, which consists a sequential combination of both displacive and disassembly/reassembly processes. We have further determined the structure of the host cell factor CypA in complex with HIV-1 capsid assembly by cryoEM. The density map unexpectedly displays a distinct non-random CypA binding pattern in which CypA bridges two adjacent CA hexamers along the curved CA array and stabilizes the viral capsid. CryoEM structure-based modeling and large scale all-atoms MD simulations surprisingly reveal that this unique CypA pattern was achieved through an additional uncharacterized novel interface so that a single CypA molecule simultaneously interacts with two CA molecules, therefore, stabilizes and protects the capsid from premature uncoating. Our structure further highlights this novel CypA and CA interface as a potentially attractive therapeutic target for pharmacological intervention.

D-106

*Structural targeting of the A32-region epitopes for antibody-dependent cell-mediated cytotoxicity*

**Marzena Pazgier, PhD**, Institute of Human Virology

Antibody-dependent cell-mediated cytotoxicity (ADCC) of non-neutralizing antibodies (nnAbs) specific to HIV envelope (Env) glycoproteins expressed on the cell surface of the target/infected cell play a role in effective adaptive immune response to HIV-1. Existing evidence points toward nnAbs recognizing the conformational CD4 inducible (CD4i) epitopes in the first and second constant (C1-C2) region of gp120 (A32-like epitopes) as important players in this process. Previously we characterized the C1-C2 region at atomic level by describing structures of several A32-like nnAbs in complexes with CD4-triggered gp120. These studies mapped the A32-epitope into mobile layers 1 and 2 of the inner domain (ID) of CD4-triggered gp120. Based on this structural information, we have developed a stable molecule expressing the C1-C2 region epitopes within a minimal structural unit of HIV-1 Env. Our construct, referred to as ID, consists of the ID of gp120 expressed independently of the outer domain and stabilized in the CD4-bound conformation by an inter-layer disulfide bond. Each phase of the design process was visualized and validated at the molecular level by structural analysis of ID variants as well as by functional testing. Our data indicate that ID expresses the C1-C2 epitopes involved in potent ADCC within the context of a CD4-triggered full-length gp120, but without the complication of other epitope regions. Thus, ID represents a novel candidate probe for the analysis and/or selective induction of antibody responses to the A32 epitope sub-region.

D-107

*Structure and Stabilization of Coronavirus Spike Proteins in the Prefusion Conformation*

**Jason McLellan, PhD**, Dartmouth College

Coronaviruses have the largest genomes among known RNA viruses and are phylogenetically divided into four genera. Some betacoronaviruses, such as HKU1, circulate annually in humans and cause mild yet prevalent respiratory disease whereas others, such as SARS-CoV and the recently emerged MERS-CoV, have caused pandemics with high case-fatality rates. Coronavirus cell tropism and host range are in large part determined by the viral surface spike (S) glycoprotein, which is the largest known class I viral fusion protein. After binding to host receptors and activation by host proteases, the S proteins undergo large conformational rearrangements that result in fusion of the viral and host-cell membranes. However, until recently, structural studies of the S proteins have been primarily limited to small protein fragments. Together with Andrew Ward and Barney Graham, we have initiated studies to provide a molecular understanding of the structure, function and antigenicity of intact, trimeric S proteins, which we believe will identify sites of vulnerability that could be targeted by vaccines, therapeutic antibodies and small-molecule antivirals. Our recent structure of the trimeric HKU1 S protein in its prefusion conformation will be presented, as well as the initial results of our structure-based vaccine design efforts.

D-108

*Structural Basis for Membrane Anchoring of HIV-1 Envelope Spike*

**Bing Chen, PhD**, Harvard University

HIV-1 envelope spike (Env) is a type I membrane protein that mediates fusion of viral and cell membranes. We have determined an NMR structure at atomic resolution of the transmembrane (TM) domain of HIV-1 Env reconstituted in bicelles that mimic a lipid bilayer. It forms a well-ordered trimer that protects a conserved arginine, buried in the membrane. An N-terminal coiled coil and a C-terminal hydrophilic core stabilize the trimer; the latter may be structurally coupled to the cytoplasmic tail. Individual mutations of conserved residues do not completely disrupt the TM trimer, and they have minimal impact on membrane fusion and viral infectivity. Major changes in the hydrophilic core, however, can alter antibody sensitivity of the functional Env. These results show how a TM domain anchors, stabilizes and modulates a viral envelope spike and suggest that its influence on Env conformation is an important consideration for HIV-1 immunogen design.

D-109

*Understanding and Exploiting the Conformational States of the HIV-1 Envelope Glycoprotein Trimer*

**Joseph Sodroski, MD**, Harvard University

Primary human immunodeficiency virus (HIV-1) envelope glycoprotein (Env) trimers typically exist in a metastable closed conformation (State 1). Binding the CD4 receptor triggers extensive conformational changes in Env, allowing Env to mediate virus entry. We investigated the conformations of the HIV-1JR-FL Env on the virus entry pathway. We identified specific gp120 residues that restrain Env in State 1. Alteration of these restraining residues destabilized State 1, allowing Env to populate a functional conformation (State 2) intermediate between State 1 and the full CD4-bound state (State 3). Increased State 2 occupancy was associated with lower energy barriers between the states. State 2 was an obligate intermediate for all transitions between State 1 and State 3. The unrestrained, State-2-enriched Envs required lower CD4 concentrations to trigger virus entry and exhibited an improved ability to infect primary macrophages. These Envs were resistant to several broadly neutralizing antibodies and small-molecule inhibitors. Thus, State 2 is an Env conformation on the virus entry pathway; sampling State 2 increases the adaptability of HIV-1 to different host cell receptor levels and immune environments. Small-molecule CD4-mimetic compounds bind gp120 and block CD4 binding. CD4-mimetic compounds prematurely trigger conformational transitions in Env, driving Env from State 1 to downstream conformations, leading to irreversible inactivation at a higher stoichiometry. At lower stoichiometry, CD4-mimetic compounds sensitize HIV-1 to neutralization and ADCC mediated by readily elicited antibodies. The potential application of HIV-1 sensitization to protection against virus transmission will be discussed.





D-110

*Real-time imaging of single HIV-1 core uncoating***Gregory Melikian, PhD**, Emory University School of Medicine

The uncoating of HIV-1 cores released into the cytoplasm as a result of viral fusion is a critical step en route to productive infection. The sites, the timing, and the extent of uncoating, defined as shedding of the capsid protein (CA) from the core complex encasing the viral genome, remain poorly understood. To elucidate this elusive step of HIV-1 entry, we developed a novel strategy to visualize the CA loss using a fluorescently tagged oligomeric form of cyclophilin A (CypA-DsRed), which binds CA with high affinity. CypA-DsRed is specifically packaged into virions and remains associated with cores after permeabilization of the viral membrane. Importantly, we show that CypA-DsRed and CA are lost concomitantly from the cores in vitro and in living cells. The rate of CypA-DsRed loss is modulated by mutations that alter the core stability and is accelerated by reverse transcription. Whereas the majority of single cores lose CypA-DsRed shortly after viral fusion, a small fraction remains intact for several hours. Single particle tracking at late times post-infection reveals a gradual loss of CypA-DsRed which is blocked upon inhibition of reverse transcription. These late uncoating events occur both in the cytoplasm and after docking at the nuclear membrane. In conclusion, the CypA-DsRed-based imaging assay enables time-resolved visualization of single HIV-1 uncoating in living cells. This novel approach provides important clues regarding spatio-temporal regulation of uncoating. This work was supported by the NIH R01 GM054787 grant and Pittsburgh Center for HIV Protein Interactions (P50GM082251).

D-111

*Coordinated gp41 and gp120 mutations conferring an open conformation of Env and their consequences on Env function***Carol Weiss, MD, PhD**, U.S. Food and Drug Administration

HIV Env undergoes conformational changes that drive virus entry and frustrate efforts to create stable immunogens. gp41 peptide fusion inhibitors interfere with conformational changes in Env in a dominant-negative manner and serve as tools to study Env conformations. Previously we found two genetic pathways of gp120 and gp41 mutations that confer cross-resistance to N and C heptad repeat peptide fusion inhibitors. Each pathway is defined by a key mutation in either the N or C heptad repeat of gp41 with accompanying gp120 mutations in CD4 binding site or V3 regions, respectively. Phenotypic studies show that mutations in the N heptad repeat greatly enhance susceptibility to sCD4 inhibition, while both pathways confer delayed entry kinetics. Sensitivity studies with a panel of monoclonals further reveal that mutations from both pathways favor Envs with more open or relaxed conformations. These studies identify residues in gp41 and gp120 that functionally interact to simultaneously influence receptor use, entry kinetics, and openness of Env conformation. The findings further highlight functional consequences of mutations that confer a more open Env structure.



D-112

*Structure of a natively-glycosylated HIV-1 Env reveals a new mode for VH1-2 antibody recognition of the CD4 binding site relevant to vaccine*

**Pamela Bjorkman, PhD**, California Institute of Technology

HIV-1 vaccine design is informed by structural studies that elucidate mechanisms by which broadly neutralizing antibodies (bNAbs) recognize/ accommodate N-glycans on the trimeric envelope glycoprotein (Env). However, variability in high-mannose and complex-type Env glycoforms leads to heterogeneity that usually precludes crystallization. We will present 3.5Å and 3.9 Å crystal structures of a native-like Env trimer with fully-processed/native glycosylation, revealing heterogeneous glycan shields of untrimmed high-mannose and complex-type N-glycans that we used to define complete epitopes of two bNAbs and potential antibody-vulnerable glycan holes. The Env trimer was complexed with 10-1074 (against the V3-loop) and IOMA, a new CD4-binding site (CD4bs) antibody. Although IOMA is derived from VH1-2\*02, the germline gene of CD4bs-targeting VRC01-class bNAbs, its light chain lacks the short CDRL3 loop that defines VRC01-class bNAbs and thus it resembles 8ANC131-class/VH1-46-derived CD4bs bNAbs, which have normal-length CDRL3s. The existence of bNAbs that combine features of VRC01-class and 8ANC131-class antibodies has implications for immunization strategies targeting VRC01-like bNAbs.

E-101

*Extracellular vesicles released by HIV-1 infected cells carry viral proteins and facilitate HIV infection of human lymphoid tissue*

**Leonid Margolis, PhD**, National Institute of Child Health and Development

It is well established that various cells in vivo and in vitro release extracellular vesicles (EVs), small phospholipid membrane-enclosed entities. Not long ago, EVs were considered to be "cellular dust" or garbage and did not attract much attention. However, they are now central to research in many fields of biology because they seem to constitute a new system of cell-cell communication. Physical and chemical characteristics of many EVs, as well as their biogenesis pathways, resemble those of retroviruses, in particular of HIV and EVs can incorporate viral components. We studied EVs generated by HIV infected cells and the effect of these EVs on HIV infection. Using tetraspanins that EVs shared with HIV, we captured both HIV virions and EVs with magnetic nanoparticles coupled to specific anti-tetraspanin antibodies and identified EVs by the presence of either CD45 or acetylcholinesterase, since virions do not incorporate these antigens. We found that approximately 30% of the EVs released by HIV-1 infected cells were positive for HIV-1 Env. Our experiments on depletion of HIV-1 preparation of EVs indicate that these EVs may facilitate HIV infection.

E-102

*Exosomes from retrovirus infected cells carry distinct viral noncoding RNAs and proteins that control the fate of the recipient cell*

**Fatah Kashanchi, PhD**, George Mason University

Recently, much interest has developed regarding mechanisms of extracellular delivery of nucleic acids and proteins among virally infected and recipient cells. While the role of exosomes in viral pathogenesis and disease states remains largely unknown, it is now widely accepted that exosomes play important roles in intercellular communication, inflammation, antigen presentation, apoptosis, and pathogenesis. We have previously reported that HIV-1 encodes its own noncoding RNA that regulates viral and host gene expression. We have recently found the presence of TAR RNA in exosomes from supernatants of HIV-1 infected cells and patient sera. We report that prior exposure of naïve cells to exosomes from infected cells increased susceptibility of the recipient cells to HIV-1 infection. TAR RNA in the serum exosomes of HAART-treated patients or LTNPs also showed  $10^3$  copies/ml. TAR is able to activate cytokines in recipient cells by increasing the nuclear accumulation of p65 and p50, related to a newly formed IKK $\beta$  complex in TAR treated cells which may be the result of TLR3, 7 and 8 activation. Using PCR, we have found a second class of viral RNAs termed "TAR/Gag" which, similar to TAR, does not translate into protein but acts as noncoding RNA. This RNA is complexed with SWI/SNF components potentially regulating HIV-1 latency in infected cells. The levels of both TAR and TAR/Gag increase in cART treated cells, indicating that viral suppression by anti-retroviral drugs will result in increased exosome release from infected cells containing viral noncoding RNAs and viral proteins. Therefore, exosomes from HIV-1 infected cART treated cells may contribute to the long lasting cytokine and immune activation observed in AIDS patients.

E-103

*Viral exosomes exert paracrine effects on endothelial cells leading to enhanced migration*

**Dirk Dittmer, PhD**, University of North Carolina School of Medicine

Paracrine signaling is a mechanism that eukaryotes have evolved to enable cell-to-cell communication, and in turn, organismal homeostasis. An emerging field of paracrine signaling is the role of secreted microvesicles, particularly exosomes, in differentiation, migration, tumor progression, and pathogen challenge. Interestingly, exosome contents are known to be modified by the cell in response to stimuli, which allows the exosomes to exert changes in neighboring cell physiology during challenge. Here we show that the tumorigenic virus Kaposi's Sarcoma-associated herpesvirus (KSHV) hijacks this paracrine signaling pathway to induce cell proliferation, migration, and inflammation in cells not infected with the virus. This signaling occurs through the canonical MEK/ERK pathway, but does not activate innate immune regulators, allowing the virus to exert these changes without cellular pathogen recognition. Collectively, we propose that KSHV establishes a niche favorable for viral spread and cell transformation through cell-derived exosomes, all while avoiding detection.



E-104

*Extracellular vesicle microRNA leads to neurotoxicity in SIV infection***Howard Fox, MD, PhD**, University of Nebraska Medical Center

To examine the role of extracellular vesicles (EVs) in HIV neuropathogenesis, we isolated and characterized EVs from the brains of rhesus macaques, both with and without SIV induced CNS disease. Small RNA sequencing revealed significantly increased miR-21 levels in EVs from SIV encephalitic brains. In situ hybridization revealed increased miR-21 expression in neurons and macrophage/microglial cells/nodules during SIV induced CNS disease. In vitro culture of macrophages revealed that miR-21 is released into EVs and is neurotoxic when compared to EVs derived from miR-21<sup>-/-</sup> knockout animals. MiR-21 alone, incorporated into EVs, is neurotoxic, and a mutation of the sequence within miR-21, predicted to bind TLR7, eliminates this neurotoxicity. Indeed miR-21 in EV activates TLR7 in a reporter cell line, and the neurotoxicity is dependent upon TLR7, as neurons isolated from TLR7<sup>-/-</sup> knockout mice are protected from neurotoxicity. We also found that EVs isolated from the brains of monkeys with SIV induced CNS disease activate TLR7 and were neurotoxic when compared to EVs from control animals. Finally, we show that EV-miR-21 induced neurotoxicity could be prevented by a necroptosis inhibitor, highlighting the actions of this pathway in a growing number of CNS disorders. The ability to isolate and analyze, molecularly and functionally, EVs from tissues such as the brain enables the study of their role in HIV pathogenesis. This work is supported by MH106422 and MH062261

E-105

*Naked virions, extracellular vesicles, and vesicle-enclosed virions released early after picornavirus infection - who, when, how, and why?***Esther N.M. Nolte-t Hoen, PhD**, Utrecht University

There is rising interest in the role of Extracellular Vesicles (EV) in virus infections. Viruses affect EV release by infected cells by influencing the incorporation of proteins and RNA from both host and viral origin into EV. Moreover, recent evidence suggests that naked viruses can exit the cell enclosed in EV (EV-virus hybrids). Enclosure of virions and viral components in EV can largely impact antiviral immunity. Still, the release kinetics of EV-virus hybrids and virus-induced EV, as well as the viral and/or host factors that regulate their formation and release, remain elusive. We addressed these topics using mengovirus, a non-enveloped, lytic RNA virus of the Picornaviridae family. A major technical hurdle in EV-virus research is the difficulty to reliably separate naked virus, (virus-induced) EV, and EV-virus hybrids due to their overlapping sizes. Our laboratory specializes in techniques to isolate subpopulations of EV, and to characterize these by general protein or RNA determination or, at the single particle level, by an in-house developed high-resolution flow cytometry (hFC) method. Using these methodologies, we discovered that early in mengovirus infection, hours before lytic release of virus, infectious particles were released from intact cells. These early stage infectious particles could be separated into populations of naked virions and EV-virus hybrids. In addition, quantitative and qualitative particle analysis by hFC indicated that virus infection induced the release of several EV subpopulations not observed in mock-infected samples. In-depth characterization of virus-induced EV and EV-virus hybrids aids our understanding of how EV contribute to viral dissemination and antiviral responses.



F-101

*Special Lecture: Programming CD8+ T Cell Immunity with Cytomegalovirus Vectors*

**Louis Picker, MD**, Oregon Health & Science University

Abstract not available.

F-102

*Special Lecture: On Death and Dying with HIV: Pyroptosis Drives CD4 T Cell Depletion*

**Warner Greene, MD, PhD**, Gladstone Institute of Virology and Immunology

Most CD4 T cells dying during HIV infection are resting "bystander" CD4 T that undergo abortive viral infection arresting during the reverse transcription (Doitsh et al., Cell 2010). IFI16-mediated sensing of the cytosolic viral DNA that accumulates in these cells (Monroe et al., Science 2014) induces inflammasome assembly and caspase-1 activation. The cells die by caspase-1 dependent pyroptosis, a highly inflammatory form of programmed cell death (Doitsh et al., Nature 2014) Recent studies demonstrate that cell-to-cell transmission of HIV-1 is required to trigger this response (Galloway et al., Cell Reports, 2015). Unlike lymphoid tissue CD4 T cells, peripheral blood CD4 T cells are highly resistant to this form of cell death due in part to a deeper resting state. Remarkably these blood CD4 T cells become sensitive to pyroptosis when mixed with a variety of lymphoid tissue cells (Munoz Arias et al., Cell Host Microbe, 2015). Pyroptosis also occurs in vivo in humanized mice acutely infected with R5-tropic HIV. Three days post-infection, the TKO-BLT animals were treated with VX-765, a selective caspase-1 inhibitor that has been shown to be safe and well tolerated in two-phase II human trials. VX-765 markedly blocked CD4 T cell depletion while not altering viral load and inhibited production of IL-18 (a caspase-1 substrate). Together, these studies highlight an important role for caspase-1 dependent pyroptosis as a major driver of HIV-associated CD4 T-cell depletion both in vitro and in vivo.

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F-103

*Structure-Function Elucidation of the Native HIV-1 Envelope Trimer As a Basis for Rational Vaccine Design*

**Paolo Lusso, MD, PhD**, National Institute of Allergy and Infectious Diseases

Envelope spikes displayed on the surface of infectious HIV-1 virions mediate functional interactions with cell-surface receptors and represent the sole target of neutralizing antibodies. Extraordinary advances have been made over the past two decades in the structural biology of HIV-1, especially through crystallization of near-native soluble trimers and high-resolution imaging of membrane-expressed trimers by cryogenic electron microscopy. Progressive refinement of the molecular anatomy of native HIV-1 envelope spikes is accruing critical information for elucidating the structural basis of specific envelope functions, such as receptor interaction and immune evasion, which may serve as a basis for rational vaccine design. Recent work in our laboratory has focused on the role of gp120 tyrosine sulfation in facilitating immune evasion, the earliest steps of CD4-trimer interaction, and the design of interdomain-locked trimers stabilized in the native configuration. These results may be instrumental for the design of improved envelope-based immunogens capable of eliciting broadly active neutralizing antibodies.

F-104

*Insights Into AIDS Virus Pathogenesis from Studies in Nonhuman Primate Models*

**Jeffrey Lifson, MD**, National Cancer Institute

Animal models can provide experimental control and flexibility that allows the investigation of questions regarding disease mechanisms not readily or feasibly pursued in a clinical setting. Nonhuman primate models have emerged as the preferred animal models for studies of AIDS virus pathogenesis. The presentation will cover recent developments in nonhuman primate models for AIDS virus studies, and insights obtained from studies employing these models.

F-105

*Vesiculovirus vectored vaccines can provide single dose protection against filoviruses, arenaviruses, and alphaviruses*

**Timothy Fouts, PhD**, Profectus Biosciences

Demetrius Matassov, PhD; Stefan Hamm, PhD; Terri Latham, BS; Becky Nowak, BS; Cheryl Kotash, BS; Daniel Colon; BS; Susan Witko, BS; Luke Jasenosky, BS; Rong Xu, PhD; Ayuko Ota-Setlik, BS; Michael Egan, PhD; David Clarke, PhD; John Eldridge, PhD

The Vesiculovirus genus, family Rhabdoviridae, is composed of negative-sense single-stranded RNA viruses. Jack Rose pioneered the development of these viruses as a vaccine platform using vesicular stomatitis virus (VSV) to express antigens from a variety of viral, protozoan, and bacterial pathogens. Further developed by Profectus BioSciences, the VesiculoVax™ vector platform is unlike adenovirus and other viral vectors as there is little pre-existing immunity that would limit vaccine take. Multiple clinical trials have shown that attenuated recombinant VSV (rVSV) vectors are safe and immunogenic in humans across a range of doses. VesiculoVax™ vectors are replication-competent, and have inherent adjuvant properties that activates innate immunity and efficiently primes and expands B cells to antibody-producing cells. This capability enables the development of prophylactic vaccines that amplify pathogen specific B-cell immune response(s) resulting in rapid induction of neutralizing antibodies and protection from disease. VesiculoVax™ based vaccines protected non-human primates against hemorrhagic fever viruses such as Ebola, Marburg, and Lassa, and is the only vaccine to have demonstrated single-dose protection of monkeys against lethal challenge with highly virulent, low-passage Ebola and Marburg viruses. VesiculoVax™ based vaccines also protected animals against chikungunya virus as well as the Western, Eastern, and Venezuelan equine encephalitic alphaviruses. An attenuated rVSV-Ebola vaccine vector has now completed enrollment in a phase 1 clinical trials. These results demonstrate that vesiculovirus vectors have tremendous potential as vaccines for protection against a wide range of acute viral infections.

F-106

*Clonally-Amplified Proviruses as Reservoirs of HIV*

**John Mellors, MD**, University of Pittsburgh

In 2014, clonal expansion of HIV-infected cells was first described as a mechanism of HIV-1 persistence on antiretroviral therapy (Maldarelli et al. and Wagner et al. Science). It was initially not known whether clonally-amplified proviruses were intact, i.e., capable of producing infectious virus. Subsequently, one case of a clonally-amplified provirus that produced infectious viremia was reported in an individual with metastatic squamous cell carcinoma (Simonetti et al. PNAS 2016). We have since performed a series of studies in individuals started on ART at various stages of HIV infection to identify other clonally-amplified proviruses, including defective and intact ones, and to characterize their integration sites, dynamics of outgrowth, transcriptional activity at the single cell level, virus production, and cell origin. Findings from these studies will be presented and their implications for HIV cure strategies will be discussed.

G-101

*Non-neutralizing antibody activities: the good, bad and indifferent*

**Donald Forthal, MD**, University of California, Irvine

Several studies have been aimed at categorizing the effect of non-neutralizing antibodies in preventing HIV infection. Some of these studies will be reviewed, and recently published and unpublished data will be described that underscore the complexity of humoral immune responses to HIV envelope glycoproteins. A particular focus will be placed on the role of non-neutralizing antibodies in enhancing infection and the potential role of phagocytosis in preventing infection.

G-102

*Fine epitope signature of HIV-1 antibody neutralization breadth at the CD4 binding site*

**Margaret Ackerman, PhD**, Dartmouth College

Major advances in donor identification and probes and methods to clone pathogen-specific antibodies have been realized over the past years, leading to exponential growth in the number of newly characterized broadly neutralizing antibodies (bnAbs) to the HIV-1 envelope, as well as to the identification of new epitopes and novel modes of antigen recognition. However, the ability to translate envelope recognition into an understanding of in vivo activity has lagged behind, and identification of blood samples or monoclonal antibodies with potent anti-viral activity has generally remained reliant on empirical evaluation of neutralization potency and breadth. Here, we undertook a study to evaluate the fine epitope specificity of a panel of CD4 binding site (CD4bs) antibodies, and in doing so, we defined the molecular recognition features of functionally potent antibodies targeting the HIV envelope CD4bs. Whereas previous studies have used neutralization data and machine learning methods to provide epitope maps, here, we reversed the process, and demonstrated that fine epitope specificity can prospectively identify broadly neutralizing CD4bs-specific monoclonal antibodies. Building on this result, we further showed that epitope mapping and effective predictions of neutralization breadth can also be achieved in the assessment of polyclonal serum responses. Bringing the discovery loop full circle, we are investigating the use of a novel probe of CD4bs neutralization breadth signature residues in identification of novel CD4bs bnAbs.





G-103

*Clonal and cellular dynamics in antibody evolution***Gabriel Victora, PhD**, Whitehead Institute for Biomedical Research

The average affinity of specific antibodies increases dramatically over the course of an immune response. This increase is the result of a Darwinian process in which B lymphocytes undergo iterative cycles of random hypermutation of their immunoglobulin genes, followed by selective proliferation of clones bearing affinity-enhancing mutations. This evolutionary process takes place in highly dynamic microanatomical structures known as germinal centers, which arise within secondary lymphoid organs upon infection or immunization. Our work combines intravital multiphoton microscopy with mouse genetics to study how the dynamics of B and T lymphocytes within germinal centers shapes the evolution of the high-affinity antibodies that are crucial to protection from infectious disease.

G-104

*Defining the earliest targets of SIV susceptibility after mucosal challenge in the Rhesus Macaque model***Thomas Hope, PhD**, Northwestern University Feinberg School of Medicine

Background: Macaque mucosal challenge via vaginal or rectal exposure with SIV is utilized to reproduce the circumstances of HIV transmission in humans. This model has provided insights into HIV transmission, but the critical window of the earliest events taking place after mucosal exposure remains undefined. Methods: We have recently developed a SIV-based dual reporter expression vector that facilitates the efficient identification of transmission susceptible sites in the rhesus macaque FRT after vaginal exposure. This system demonstrated that initial infection events can be widespread throughout the female reproductive tract (FRT), highly variable in their localization, and that T cells are the primary target in initial infection. We have extended this approach in two ways to gain additional insights into the earliest aspects of mucosal transmission. First, after rectal challenge with the SIV-based dual reporter expression vector, we can identify the location and phenotype of the cells infected by the inoculum of exposure. Additionally, because this system efficiently identifies regions of susceptibility to infection in the FRT, we have determined that we can identify small foci of SIVmac239 infection 48 hours after vaginal challenge with a mixture of wildtype SIVmac239 and the LIC dual reporter. Utilizing this novel approach to SIV challenge, we routinely identify SIVmac239 infected cells revealing their localization and fates in the FRT 48 hours after vaginal challenge. Results: Foci of infection with SIVmac239 are found throughout the female reproductive tract, from labia to ovary. We find that T cells are the major targets, and there is a strong bias for those with a Th17 phenotype. Infection of immature dendritic cells and macrophages is also observed representing approximately 25% of infected cells. Initial studies after rectal challenge also reveal that Th17 cells and immature dendritic cells are the primary target cells susceptible to the initial inoculum. Unexpectedly, we find that transmission can also take place in anal tissue which is protected by a squamous epithelium similar to the vaginal vault. Conclusions: Defining the location and phenotype of cells susceptible to SIV infection after vaginal or rectal challenge informs the development of interventions designed to decrease HIV acquisition. We find that there is great similarity relating to the initial targets of infection by the inoculum of exposure. In both cases, we find preferential infection of Th17-like cells and immature dendritic cells in contexts protected by both squamous and columnar epithelial mucosal barriers. Both of these cell types are involved in continuous immune surveillance at mucosal surfaces and could partially explain the known conditions that increase HIV acquisition, including sexually transmitted infections and bacterial vaginosis. How these conditions precisely influence mucosal barrier function or the density of target cells remains to be determined. However, the system presented here provides the ability to study and sample these foci at the portal of entry, facilitating the ability to characterize the earliest host responses to SIV/HIV infection.

G-105

*HIV Vaccines Based on Transition State Envelope Structures*

**Anthony DeVico, PhD**, Institute of Human Virology

Protective efficacy from an HIV vaccine will heavily depend on anti-envelope antibodies that block the entry and spread of diverse viral strains. Thus, an HIV vaccine will have to generate humoral responses that focus on highly conserved and functional epitopes and thereby mediate their antiviral effects. Accordingly, we have been developing HIV vaccine strategies based on observations that very highly conserved epitopes are presented on HIV gp120 as it transitions through different structural states during viral entry. Here we will describe progress in the clinical development of an immunogen (FLSC) designed to mimic transition state gp120 structures along with new imaging data that elucidates potential virological explanations for vaccine effects.

G-106

*Mucosal vaccination with a replication-competent VSV-HIV chimera delivering Env trimers protects rhesus macaques from rectal SHIV infection*

**Christopher Parks, PhD**, International AIDS Vaccine Initiative

Multiple licensed vaccines are based on live attenuated enveloped viruses. Because these vaccines cause a mild infection, they effectively direct immune responses against authentic viral targets, including multimeric transmembrane glycoproteins arrayed on the surface of infected cells and virus progeny. To develop a vaccine that would mimic transmembrane Env presentation that occurs during an HIV infection, we used vesicular stomatitis virus (VSV) to generate a replication-competent VSV-HIV chimera (VSVΔG-EnvG) in which the native VSV glycoprotein (G) was replaced with subtype A HIV Env from strain BG505. Ten Indian rhesus macaques were vaccinated with VSVΔG-EnvG by applying live vaccine to intranasal and intraoral surfaces after which all animals developed Env antibodies. Seven of 10 vaccinated macaques were protected from 10 sequential intrarectal challenges with heterologous subtype B SHIV SF162p3 while 9 of 10 unvaccinated controls became infected resulting in 67% efficacy (P=0.014). Although significant HIV pseudovirus neutralization activity was not detectable in serum from vaccinated macaques, protection was associated with Env-specific binding antibodies. Importantly, contrasting results were produced in a third study group vaccinated with a different VSV vector (VSV-G6-EnvG), which expressed the same Env immunogen as well as G. VSV-G6-EnvG also elicited Env antibodies but failed to induce protective immunity. Neither VSV-based vaccine evoked a strong anti-Env cellular immune response detectable in peripheral blood. These results indicated that protection from SHIV infection induced by VSVΔG-EnvG was associated with Env-specific binding antibodies but not T cells. Furthermore, the differing efficacy of the two types of VSV-based vaccines indicated that vector design significantly modulated the qualities of the polyclonal antibody response.

G-107

*High Resolution of Humoral Responses to HIV-1: determinism or chance?*

**Garnett Kelsoe, DSc**, Duke University School of Medicine

HIV-1 and AIDS are threats not only to the health of individuals but to societies and nations. While a vaccine that induces broadly neutralizing antibodies (bNAbs) against HIV could be transformative for intervening in the HIV-1 pandemic, no vaccine has been shown to induce HIV-1 bNAbs and while the somatic evolution of bNAb development has been mapped, we do not yet understand the origins of bNAb responses and why they arise infrequently. To be effective, HIV-1 vaccine antigens must carry neutralizing epitopes recognized by bNAb lineage founders. In addition, it may also be necessary to activate B cells that escape from limiting mechanisms of immune control. Indeed, atypical characteristics of bNAbs, including high frequencies of somatic mutations and frequent poly- or autoreactivity, suggest that the induction of bNAb responses may be problematic even with ideal immunogens. Consequently, we have begun to characterize – on a single-cell basis - humoral immune responses elicited in rhesus macaques (RMs) by novel SHIV strains that establish chronic and high viral-load infections. We have focused on the germinal center and memory B-cell compartments and established high-throughput methods capable of detailing even complex humoral responses. In this way, we hope to overcome a major limitation in our knowledge of HIV-1 bNAb responses, namely that we entirely rely on rare natural histories, observational and correlational data, of B-cell populations that arise in individual patients infected in different ways by different viruses. The primary question that we are asking is straightforward: how similar are humoral responses of individual RMs to identical SHIV infections? The answer to this simple, but neglected question is crucial to the design of effective vaccine strategies.

G-108

*Special Lecture: HIV-1 Prevention: Progress Towards Passive or Active Vaccination*

**Michel Nussenzweig, MD, PhD**, The Rockefeller University

AIDS is a preventable disease. Nevertheless, 2.1 million individuals were infected worldwide in 2015 and therefore an effective vaccine would be highly desirable. Most vaccines in clinical use today prevent infection because they elicit antibodies that block pathogen entry, and studies in experimental animals suggest that pre-existing broadly neutralizing antibodies to HIV-1 can prevent infection. However, despite a significant effort by numerous investigators over the last 30 years eliciting potent antibodies with neutralizing breadth against HIV-1 by vaccination has not been possible. Recent progress toward the goal of active or passive HIV-1 vaccination will be summarized.

G-109

*Special Lecture: Engineering anti-HIV antibodies for optimal control of HIV infection*

**Jeffrey Ravetch, MD, PhD**, The Rockefeller University

Broadly neutralizing antibodies (bNAbs) against the envelope glycoprotein of HIV-1 (Env) suppress viremia in animal models of HIV-1 and humans. To achieve potent activity without the emergence of viral escape mutants, co-administration of different bNAbs is necessary to target distinct, non-overlapping epitopes essential for viral fitness. In addition to Fab mediated neutralizing activity, Fc effector activity resulting from selective FcγR binding is required to mediate clearance of viral particles, elimination of infected cells and induction of cellular responses. I will discuss the development and evaluation of new classes of anti-HIV antibodies, in which engineering of the Fab, hinge and Fc domains has resulted in antibodies with remarkable breadth, potency, half-life and effector activity. Bispecific anti-Env neutralizing antibodies (biNAbs) with potent in vitro and in vivo activity was achieved by engineering the hinge domain of IgG1 to increase Fab domain flexibility necessary for hetero-bivalent binding to the Env trimer. Compared to unmodified biNAbs, hinge domain variants exhibited substantially improved neutralization activity, with particular combinations showing evidence of synergistic neutralization potency in vitro and enhanced in vivo therapeutic activity in HIV-1-infected humanized mice. Combining these biNAbs with Fc's modified to enhance FcRn and FcγR binding has resulted in molecules with extended half-life, enhanced effector activity. These findings suggest innovative strategies for generating anti-HIV antibodies with remarkable neutralization breadth, potency, half-life and effector activities, representing ideal candidate molecules for the control of HIV-1 infection.

G-110

*Events in Early HIV-1 Infection That Prime the Development of Heterologous Neutralization Breadth*

**Cynthia Derdeyn, PhD**, Emory University School of Medicine

A recent IAVI study reported that viral load, HLA-A\*03, and subtype C HIV-1 were strongly associated with the development of neutralization breadth in a multi-site African cohort. Here, we investigated the impact of early autologous neutralization and envelope (Env) diversification in subjects in Kigali, Rwanda (n=9) and Lusaka, Zambia (n=12), in conjunction with correlates from the parent cohort. Our analysis revealed that potent neutralization against the transmitted/founder (T/F) Env and extensive Env diversification leading to viral escape within 4-8 months from infection were vital components for the development of breadth, demonstrating priority over the population-based correlates. To gain further insight into how early antibodies against the T/F Env influence breadth, we characterized 75 monoclonal antibodies (mAbs) from the top neutralizer and 74 mAbs from a poor neutralizer. The mAbs were recovered from single memory B cells tagged by the autologous T/F gp120 protein. Examination of germline usage revealed that the anti-gp120 mAbs in both individuals (and in 2 others) utilized numerous heavy chain germline lineages that have been associated with bnAb activity, suggesting that these are generic responses. mAbs from the poor neutralizer were significantly more clonal, more somatically mutated, had longer CDRH3s, and bound with higher affinity (KD) to the autologous T/F gp120 protein than those from the top neutralizer. Together, these studies highlight the complexity of the development of neutralization breadth from early autologous antibodies during HIV-1 infection, and challenge the concept that activation of a particular germline or driving common bnAb-associated attributes will necessarily lead to bnAbs.



G-111

*Identification of Human Long-lived Plasma Cells: Implications for HIV Vaccines*

**Frances Eun-Hyung Lee, MD**, Emory University School of Medicine

Antibody responses to viral infections can be sustained for hundreds of years by long-lived plasma cells (LLPCs). The goal of any immunization is to maintain durability of serum antibody responses by the generation of LLPCs. However, LLPCs had yet to be characterized in humans. Here we used CD19, CD38 and CD138 to identify 4 distinct PC populations in the human bone marrow (BM). We show that the CD19-CD38hiCD138+ fraction is morphologically distinct and represents the exclusive repository of PCs specific for viral antigens to which the subjects had not been exposed for more than 40 years. We also show that protein sequences of viral-specific circulating antibodies are encoded exclusively by the BM CD19-CD38hiCD138+ PCs. Reconstitution of the monoclonal antibody from the BM PC RNA sequence demonstrates virus specificity. Additionally, Next Generation Sequencing (NGS) identifies a distinct VH repertoire of the CD19-CD38hiCD138+ subset that is relatively uncoupled from other BM PC subsets, suggesting that this compartment represents the B cell response's "historical record" of antigenic exposure. Combined, our studies provide original evidence for a bone fide, discrete long-lived plasma cell compartment within the human BM and identify the ideal human PC compartment to generate vaccine-specificities including the HIV broadly neutralizing antibodies (HIV BNA).

G-112

*The effect of stress agents in vitro and human vaccination in vivo on stem cell memory CD4+ CD45- T cells*

**Thomas Lehner, MD**, King's College London

The long-term efficacy of vaccines is dependent on immunological memory of T and B cells. Recent advances in CD4 CD45RO+ memory T cells have identified CD4+ CD45RO- stem cell-like memory cells (SCM) in murine and human T cells. The objectives of this investigation were to establish if SCM cells are induced by vaccination in the RV144 clinical trial. We report here that ALVAC/AIDSVAX B/E induced significant increase in CD4 SCM, with phenotypic expression of CD45RO- CD62L+ CCR7+ CD95+ SCM (p=0.004). Characterisation of two SCM sub-types 28 weeks after vaccination revealed significant increase (p<0.002) in cells expressing CD122 (the  $\beta$  chain of IL-2/IL-15 receptor), whereas SCM expressing CCR5 (HIV-1 co-receptor) were significantly decreased (p<0.005). These inverse changes were also seen in CD4 CD45RO+ CCR7+ central but not in CD45RO+ CCR7- effector memory T cells. The CXCR4 expressing SCM or the placebo controls showed no change. Furthermore, recombinant IL-15 induced replication of SCM, and oxidative stress agents elicited membrane associated (ma) IL-15, which also induced significant proliferation of SCM. Importantly, both CD4+ CD45RO- SCM and CD4+ CD45RO+ CCR7+ central memory T cells can harbour latent HIV-1. The data suggest the paradigm that increase in CD4+ CD122+ SCM and central memory T cells will boost long-term memory and simultaneously decrease in HIV-1 binding of corresponding CCR5+ cells will reduce HIV-1 infectivity. The effect on latency of the two major cell subsets harbouring latent HIV-1 will be studied.

H-101

*Introduction to Lifetime Achievement Awards*

**Robert C. Gallo, MD**, Director, Institute of Human Virology

H-102

*Speaking in honor of Peter Vogt: Pseudoviruses: Sheep in Wolves' Clothing*

**Robin Weiss, MD, PhD**, University College London

Pseudoviruses or pseudotypes possess the core and genome structure of one virus and the envelope glycoproteins of another. The term was first used for envelope-defective strains of Rous sarcoma virus bearing leukemia virus envelopes, pioneered by Peter Vogt and Hidesaburo Hanafusa. Pseudoviruses later led to the development of retroviral vectors for gene transfer. Retroviruses and lentiviruses expressing cloned envelope genes have proved useful for determining cell surface receptors for viruses, and also for measuring the titer and breadth of neutralizing antibodies. Vesiculo-stomatitis virus pseudotypes were pioneered by Jan Zavada and modern forms look promising as replication-competent yet apparently safe vaccines, eg. against Ebola. The assembly of pseudoviruses bearing the envelopes of highly pathogenic viruses provides a safe and simple means for titrating the neutralizing properties of sera and monoclonal antibodies derived from patients naturally infected with enveloped viruses such as HIV, H5N1 Influenza, Rabies and Ebola, as well as antibodies elicited by candidate vaccines.

H-103

*Speaking in honor of Peter Vogt: The Epstein-Barr Virus, a 50 year Odyssey*

**Joseph Pagano, MD**, University of North Carolina, Professor of Medicine and Microbiology and Immunology, Lineberger Professor and Director Emeritus, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

The Epstein - Barr Virus (EBV), is the first human tumor virus identified and is strongly associated with endemic Burkitt's Lymphoma (BL) in Africa. It is causative agent of the B-cell lymphomas that arise in innate and acquired immunocompromised persons. It is also causative in hairy leukoplakia and infectious mononucleosis. Notably, it is almost certainly causative in the malignancy that originates in epithelial tissue in Waldeyer's Ring in the Fossa of Rosenmuller of the posterior nasopharynx (NPC). EBV is invariably present in NPC world-wide, whether sporadic or endemic in incidence.

We focused first (1971) on detection of EBV DNA in BL tissue with a highly sensitive cRNA-DNA assay that permitted precise quantitation of number of EBV genomes in tissue extracts.

Later we used this probe for in-situ hybridization studies in BL cells and also in NPC tissues, as well as for detection of EBV in oropharyngeal epithelial cells obtained from students with infectious mononucleosis. The findings also showed that EBV replicated initially in normal human epithelial cells.

Additionally we recognized earlier that the EBV genome could exist in cells in supercoiled episomal form, as well as the linear format found in virus. The EBV episome is the molecular basis for latent EBV infections.

Achievements that followed were characterization of the viral DNA polymerase and understanding its interaction with antiviral drugs. Studies with new antiviral drugs are continuing.

The landmark discovery of IRF7 (1997), the seventh human interferon regulatory factor discovered, was later recognized as the essential common regulator of the expression of Type 1 interferons.

Mechanisms of induction of invasion, metastasis and angiogenesis induced by the principal EBV oncoprotein, LMP-1, have now spanned 20 years and remain a main focus.

Current work addresses ubiquitin systems that involve EBV, in particular its deubiquitinating enzyme, and its involvement in mechanisms of DNA repair, EBV immortalization of B-cells, and the genesis of human B-cell lymphomas in humanized mice..

The fascinations of EBV and how it works continue!

H-104

*Special Lecture in honor of Peter Vogt: Novel infectious agents in dairy cattle and their role in human chronic diseases*

**Harald zur Hausen, MD, Nobel Laureate**, German Cancer Research Center

Novel Infectious Agents in Dairy Cattle and their Potential Role in Human Chronic Diseases Harald zur Hausen and Ethel-Michele de Villiers, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany Geographic epidemiology of colon and breast cancers points to red meat and milk products derived from dairy cattle as potential risk factors for these cancers (zur Hausen and de Villiers, 2015, zur Hausen 2015). The analysis of sera and dairy products from dairy cows resulted in the isolation of 22 novel virus-like single-stranded circular DNAs which range in size between 1084 and 2958 nucleotides. They seem to persist episomally and represent members of three different families. Two of the latter contain an open reading frame related to a bacterial plasmid. Transfection experiments have been performed in human cells in order to exclude bacterial contamination as origin. All genomes which have been tested up to now are genetically active in human cells. Characterization of the transcribed RNA points to adaptation of these agents to mammalian cells. Presently, their protein expression and host reactivity to expressed proteins are being analyzed. The isolation of two of these agents from lesions of patients with multiple sclerosis is also directing our interest to a potential involvement of some of these agents in neurodegenerative diseases. References: zur Hausen, H. and de Villiers, E.M. Dairy cattle serum and milk factors contributing to the risk of colon and breast cancers. *Int J Cancer*. 2015; 137: 959-967 zur Hausen, H. What do breast and CRC cancers and MS have in common? *Nature Rev. Clinical Oncology*, 2015; 12: 569-70.

H-105

*Reinhard Kurth Memorial Lecture: The Non-coding Transcriptome: Regulation by MYC and Cancer-specific Transcripts*

**Peter Vogt, PhD**, The Scripps Research Institute, The 2016 IHV Lifetime Achievement Award for Scientific Contributions

We have identified a small molecule inhibitor of MYC. The compound inhibits the MYC transcriptional program and is effective in blocking the growth of MYC-driven xenotransplants. The characterization of this inhibitor led to the discovery that MYC regulates a large proportion of the non-coding transcriptome. Cellular levels of MYC affect virtually all non-coding transcripts either positively or negatively. MYC binds to promoter-proximal sites of non-coding transcripts and therefore appears to act directly, not through the agency of a subordinate transcriptional regulator. This effect of MYC on the non-coding transcriptome has been confirmed by RNAseq, qRT-PCR and nuclear run-on and is in accord with global epigenetic and ChIP data. An important and still poorly understood feature of this regulatory action of MYC is that MYC binding to transcriptional start sites is identical across different cell lines, but the resulting expression patterns are cell line-specific. The factors involved in this specificity have not been identified. The regulation of the non-coding transcriptome by MYC constitutes a huge expansion of MYC activity and amounts to multiplying the MYC universe. Because of the oncogenic potential of MYC, we investigated non-coding transcriptomes for cancer-specific lncRNAs. We identified several such lncRNAs, including one that is specific for luminal breast cancer, is exported from the cancer cells by an exosomal mechanism and is taken up by surrounding, non-expressing cells. Functional characterizations of lncRNAs that are specific for a certain cancer type are in progress.

H-106

*Speaking in honor of Ray Schinazi: Assessing the contribution of myeloid cells to HIV-1 persistence in the face of ART*

**Mario Stevenson, PhD**, University of Miami Miller School of Medicine

Antiretroviral therapy (ART) can sustain suppression of plasma viremia for years. However, if therapy is interrupted, there is a rapid resumption in viremia. Therefore, HIV-1 has the ability to persist in the face of potent ART. Identifying the mechanism through which HIV-1 persists in infected individuals on suppressive therapy is central to the goal of curing infection in these individuals. For this reason, research is focused on establishing tools with which to probe the reservoirs that persist in aviremic individuals as well as clinical protocols with which to perturb those reservoirs. How we measure viral reservoirs in aviremic patients and, as a consequence, how we gauge the impact of treatments on those reservoirs is a contentious issue. Most attention has focused on the role of latently infected T-cells in viral persistence and clinical strategies are geared towards purging of these reservoirs. However, other factors may be contributing to viral persistence including residual viral replication and establishment of non-T-cell reservoirs. In addition, although ART significantly improves health outcomes for the patient, several markers of immunopathogenicity persist in the face of effective viral suppression and drive co-morbidities. Studies examining the potential role of myeloid cells to viral persistence in the face of suppressive ART will be discussed.





H-107

*Speaking in honor of Ray Schinazi: Thirty Years of Anti-Retroviral Therapy for Patients with AIDS***Samuel Broder, MD**, Intrexon

We are approaching the thirtieth anniversary of the FDA approval of AZT. This was the first therapeutic agent targeting HIV-1, the retrovirus responsible for the Acquired Immunodeficiency Syndrome (AIDS) that was approved. In that time, AIDS has gone from being an “inherently untreatable” infectious agent to one eminently susceptible to a range of therapies. During a five-year period, starting in the mid-1980s, my group at the National Cancer Institute played a unique and foundational role in the discovery and development of the first generation of antiretroviral agents. We initially focused on AZT and related congeners in the dideoxynucleoside family of nucleoside reverse transcriptase inhibitors (NRTIs), taking them from the laboratory to the clinic in response to the pandemic of AIDS, then a terrifying and lethal disease. Starting with AZT, these drugs proved, above all else, that HIV-1 infection is treatable, and such proof provided momentum for new therapies from many sources, directed at a range of viral targets, at a pace that has rarely, if ever, been matched in modern drug development. Antiretroviral therapy has brought about a substantial decrease in the death rate due to HIV-1 infection, changing it from a rapidly lethal disease into a chronic manageable condition, compatible with very long survival. This has special implications within the classic boundaries of public health around the world, but at the same time, in certain regions, may also affect a cycle of economic and civil instability in which HIV-1/AIDS is both cause and consequence. Many challenges remain, including 1.) lifelong duration of therapy; 2.) implementation of pre-exposure prophylaxis (PrEP); 3.) care coordination and case management for viral suppression in substance-using patients; 4.) risk of within-couple, condomless sexual activity when the HIV-1 positive partner is on suppressive antiretroviral treatment; 5.) cardiometabolic side effects or other toxicities of long-term therapy; 6.) emergence of drug-resistance and viral genetic diversity (non-B subtypes); 7.) the specter of new cross-species transmissions from established retroviral reservoirs in apes and Old World monkeys; and 8.) the continued pace of new HIV-1 infections in many parts of the world. All of these factors make refining and surpassing current therapies, and developing new therapeutic paradigms, essential priorities.

H-108

*Special Lecture in honor of Ray Schinazi: The HCV Story: From Origins to Cure***Harvey Alter, MD**, National Institutes of Health

First recognized in 1975, the non-A, non-B agent was cloned in 1988 and renamed the hepatitis C virus (HCV). HCV is now known to be the world’s leading cause of cirrhosis and hepatocellular carcinoma and leading indication for liver transplantation. It is estimated that there are 3-5 million carriers of HCV in the US and 150 million worldwide. Although only 30-40% of carriers progress to these severe outcomes, the high HCV prevalence results in an enormous global disease burden. Approximately 75% of acutely infected persons become persistently infected. Persistence is not due to viral integration, but to blunting the innate and adaptive immune response by viral encoded proteins and, like HIV, to a constantly evolving viral quasispecies. Interferon (IFN) based therapies were at most 50% effective. Newly developed HCV-specific drugs, direct acting antivirals (DAAs), first targeted to the viral protease (NS3) and when combined with IFN increased the sustained virologic response rate (SVR), tantamount to cure, to 70%. To circumvent the use of IFN, DAAs were directed to other genomic regions, particularly the NS5B polymerase. A major breakthrough occurred when the Schinazi lab targeted the NS5A replication complex and developed a potent inhibitor. Ultimately, Gilead combined NS5A and NS5B inhibitors into a single pill taken once daily for only 12 weeks that provided a >90% SVR; a second generation, pan-genotypic version has shown 98-99% SVR. It has been shown that SVR can halt the progression to cirrhosis and diminish the incidence of HCC. It is now conceivable that HCV infection could be eradicated even in the absence of a vaccine. However, dissemination of these “miracle” drugs has been severely limited by high cost. Even at their high costs, DAAs have been calculated to be cost-effective, but neither third party payers nor governments have been willing to absorb the large upfront costs. There is need for a treatment model similar to that for HIV. There have been many heroes in the NANB/HCV story; Ray Schinazi whom we honor stands out among them.



I-101

*Public Health Approach to HIV Stagnation***John Bartlett, MD**, Johns Hopkins University School of Medicine

Progress in HIV care since the report of triple therapy in 1996 is undeniable and we now speak frequently of cure and vaccine. The reality is that despite good treatment and prevention, there continues to be about 50,000 new cases/year, about 14% are undiagnosed, and those infected have a lifetime of meds. Tom Frieden, Director of the CDC, notes the great contribution of failed communication between experts in public health and those doing primary HIV-care: different thought leaders, journals, meetings and funding streams. His plea is to focus efforts on two groups that account for the vast majority of new cases: young MSM and patients who had fallen out of care. The former group needs aggressive HIV testing—preferably NAT testing to detect acute infection especially when most contagious; the latter group needs appropriate incentives (\$) to retain patients in care. An example using \$1,000/yr/patient would be enormously cost effective. Other methods to be suggested include: use of encrypted social media to improve compliance, use of new long acting ART and implementation of PrEP which has performed well in trials but failed with implementation. The bottom line in this effort includes a novel collaboration between HIV public health experts in care providers to rethink the way we do care and pay for care.

I-103

*The Absence of Drug Resistance against Dolutegravir in First-Line Therapy is Attributable to Reduced Viral Replicative Fitness***Mark Wainberg, MD**, McGill University

Background and Methods: Dolutegravir (DTG) is an integrase strand transfer inhibitor (INSTI) against which drug resistance in first-line therapy has never been observed. However, a R263K mutation that confers low-level resistance (3-4 fold) to DTG was selected by us in culture and also developed in several patients who received DTG as an INSTI after having failed other drugs. The absence of resistance to DTG is due to a high fitness cost that is exacted by the R263K mutation and the fact that compensatory mutations for R263K have not occurred. We measured levels of integrated HIV DNA in cells infected by HIV containing R263K and other INSTI and non-INSTI resistance mutations. Results: The R263K substitution alone conferred an approximate 3-fold level of resistance to DTG, a 40% loss in viral replicative capacity and a 40% drop in recombinant integrase activity. A continuation of DTG drug pressure led to secondary mutations at positions H51Y, E138K, or T66I that did not individually affect DTG resistance or enzyme activity. However, the combination of R263K with H51Y or E138K slightly increased DTG resistance but also caused a ≈90% loss in each of viral replication capacity and integrase activity as measured both biochemically and by PCR. Most important, the continued propagation in culture of viruses containing both R263K and H51Y yielded progressively less integrated viral DNA in successive infections, beginning at ≈30% of wild-type and dramatically decreasing to non-detectability thereafter. In addition, our data show that HIV that is subjected to DTG pressure is unable to evolve and remains durably susceptible to anti-HIV immune responses. Conclusions: Our findings explain why drug resistance to DTG has not been observed after first-line therapy for more than three years since its approval by regulatory agencies. The use of DTG in first-line therapy may be compatible with treatment interruption strategies aimed at attaining a functional HIV cure because of the non-development of drug resistance.



I-104

*Metabolic and cardiovascular co-morbidities in people living with HIV***Barry Peters, MD**, King's College London

Effective antiretroviral therapy has resulted in a marked reduction of HIV associated opportunistic disease but has revealed an increased propensity of people living with HIV (PLWH) to suffer from non-AIDS conditions. A large proportion of these conditions are those associated with aging, including cardiovascular disease, diabetes, and osteoporosis. Type 2 diabetes, as an example, is growing in incidence worldwide due to increased longevity and changes in modifiable risk factors, such as diet. HIV might add a further layer of risk, and we have found estimates of prevalence of T2D in HIV that vary from between 2.6 and 14%. This range is likely to reflect the populations and groups studied, and differences in their traditional risk factors for T2D, as well as additional risk factors for T2D, including duration of HIV infection, degree of immunosuppression, and exposure to those ARVs known to be associated with insulin resistance. Similarly, reduced bone mineral density and increased incidence of osteoporosis and the increased incidence of cardiovascular disease are associated with certain ARTs and with HIV itself. PLWH with T2D have poorer reported outcomes and hence early identification of pre-diabetes will enable preventative measures to be introduced, and will mitigate against the need to manage T2D subsequently. The increased incidence of these co-morbidities requires appropriate research into the aetiology, associations and management of these conditions in order to reduce future morbidity in people living with HIV infection.

I-105

*Advances in the treatment of chronic HCV infection with direct acting antivirals***Mark Sulkowski, MD**, Johns Hopkins University School of Medicine

Next generation of DAAs are aimed at 1) Person who need "salvage" regimens that can overcome HCV drug resistance associated variants (RAVs); 2) HCV genotype 3; 3) Persons for whom ribavirin is still recommended. HCV NS3 Protease inhibitors. The first wave of HCV protease inhibitors were not pangenotypic, lacking potent antiviral activity against HCV genotype 3. There are two protease inhibitors in phase 3 clinical trials that are once daily, potent, pangenotypic drugs: Voxilaprevir (VOX, GS-9857) and ABT-493. Voxilaprevir is being developed as a fixed-dose combination tablet including sofosbuvir and velpatasvir (a pangenotypic NS5A inhibitor). ABT-493 is being developed as a fixed-dose combination tablet including ABT-530 (a pangenotypic NS5A inhibitor). HCV NS5A inhibitors. Similar to protease inhibitors, many of the first wave of NS5A inhibitors lacked activity against HCV genotype 2 and 3 infection (the exception is daclatasvir). Velpatasvir is pangenotypic and has completed phase 3 trials in combination with sofosbuvir. The fixed-dose combination tablet given for a duration of 12 weeks led to HCV cure in > 99% of persons with HCV genotype 1, 2, 4, 5 and 6 infection and 95% of those with HCV genotype 3. This regimen is expected to be approved in the US and Europe in 2016. Other pangenotypic protease inhibitors in development include MK-8408 and the above-referenced ABT-530. While studies are ongoing, ABT-530 appears to be among the most active NS5A inhibitors against NS5A RAVs are position 93. HCV NS5B inhibitors. To date, sofosbuvir is the only approved nucleotide analogue NS5B inhibitor. Other DAAs in this class have been discontinued due to drug toxicity. In this context, MK3682 and AL-335 are currently in phase 2 clinical trials. MK3682 is being developed in combination with grazoprevir and MK-8408 as a fixed dose combination tablet for the treatment of all HCV genotypes. Similarly, AL-335 is being tested in combination with Odalasvir (NS5A inhibitor), and Simeprevir for the treatment of Genotype 1 chronic HCV infection.



I-106

*Special Lecture: Transforming anti-HIV drugs***Howard Gendelman, MD**, University of Nebraska

Our work focuses on transforming existing anti-human immunodeficiency virus (HIV) drugs into potent long-acting nanoformulated antiretrovirals (nanoART) together with the development of agents that boost drug depots by affecting autophagy. The sustained release products (SRP) were shown in our prior works to attenuate viral infection with dosing intervals of once a month or longer. The chemical process involves conversation of hydrophilic antiretroviral drugs (for example entry, nucleoside and nonnucleoside reverse transcriptase inhibitors) into crystalline hydrophobic prodrugs. Drug encapsulation into decorated (cell targeted) nano particles are carried in mononuclear phagocytes (MP: monocytes, perivascular and tissue macrophages) autophagosomes. The end result are anti-HIV SRPs with prolonged half-lives. Drug pharmacokinetics (PK) are governed by rate of antiretroviral drug (ARV) crystal particle dissociation and drug hydrolysis. This directive can circumvent drug toxicities, improve regimen compliance and facilitate penetrance into viral reservoirs [notably gut, lymphoid organs and the central nervous system]. Reduction in residual infection was shown through pharmacodynamic tests. MPs are the depots and enabler of drug transport to tissue reservoirs. The ARV particles can readily be delivered to subcellular sites of viral replication. The goal of ARV transformation into SRP products will be step-wise. The first part rests in making the drug libraries. The second encases prodrugs into decorated poloxamers. The third characterizes the SRPs to optimize its size, shape, polydispersity and particle integrity. Antiretroviral responses and drug endosomal trafficking will be discussed. Our use of SRP boosting agents for sustaining intracellular depots with autophagy drugs that facilitate particle autophagosomal depots have been shown to improve ARV biodistribution. Discussion of "state of the art" tools to improve nanoART access for human use will be discussed.

I-107

*Challenges in eradicating chronic HBV infection***Shyamasundaran Kottitil, MD, PhD**, Institute of Human Virology

Chronic hepatitis B infection affects >300 million people worldwide and is a leading cause of liver failure and cancer. Current approaches to treatment for chronic hepatitis B involve suppression of hepatitis B virus (HBV) DNA with the use of nucleoside analogues. Chronic suppressive therapy rarely results in a "functional cure" or absence of detectable HBV DNA in plasma and loss of detectable hepatitis B surface antigen after cessation of therapy. The major obstacles to achieving a functional cure are the presence of covalently closed circular DNA and ineffective/exhaustive immune system. This presentation focuses on novel approaches to target viral life cycle and host immunity to achieve a functional cure.





I-108

*Discovery to first-in-man of a multi-peptide-based hepatocellular carcinoma vaccine adjuvanted with CV8102 (RNAdjuvant) - HEPAVAC*

**Luigi Buonaguro, MD**, National Cancer Institute "Fondazione Pascale"

HCC/normal adjacent tissue matched samples have been collected for HLA immunopeptidome analysis. 17 HCC samples from HLA-A\*02+ patients and 15 samples from HLA-A\*24+ patients have been analysed by mass spectrometry (LC-MS/MS). A total of 16 epitopes have been selected for the HepaVac vaccine and are currently synthesized according to GMP standard. Of these, 7 are restricted to HLA-A\*02; 5 HLA-A\*24 and 4 HLA class II. Formulation development studies have been undertaken leading to a suitable and stable pharmaceutical form. An analytical method was developed which allows the characterization of each individual epitope within the HepaVac vaccine (IMA970A). A single-arm, first-in-man trial entitled HepaVac-101 is designed to investigate the off-the-shelf, multi-peptide-based HCC vaccine (IMA970) plus CV8102 (RNAdjuvant an immunomodulator) following a single pre-vaccination infusion of cyclophosphamide (acting as immunomodulator) in patients with very early, early and intermediate stage of HCC. The primary endpoints are safety and tolerability, and immunogenicity. Secondary/exploratory endpoints are additional immunological parameters in blood (e.g. regulatory T-cells, myeloid-derived suppressor cells, impact of the standard therapy on the natural immune response), infiltrating T-lymphocytes in tumor tissue, biomarkers in blood and tissue, disease-free survival/progression-free survival and overall survival. Once safety of this vaccination approach has been determined in the first 10-20 patients the addition of a checkpoint inhibitor will be considered. Suitable patients enrolled in Tuebingen are invited to participate in an extension investigating an actively personalized vaccine (APVAC) plus CV8102. The HepaVac project started in September 2013 and is supported by the European Commission's 7th Framework Program under the Grant Agreement Nr. 602893 ([www.hepavac.eu](http://www.hepavac.eu)).

I-109

*Transplantation in the HIV Positive Recipient: The Unexpected Findings*

**Peter Stock, MD**, University of California, San Francisco

Decrease the rejection rates include the utilization of antiviral regimens which do not impact the cytochrome p450 system to avoid the drug to drug interactions between immunosuppression and antiretroviral agents. An interesting strategy currently being tested in a clinical trial will determine the impact of CCR5 blockade in blocking the immune response and decreasing HIV persistence. Other interesting and unexpected findings include the impact of TOR inhibitor on depleting HIV persistence in the CD4+ lymphocytes. Serendipitous events in our initial trials have revealed immunosuppressive agents with anti-retroviral qualities (sirolimus/TOR inhibitor), and antiretroviral agents (CCR5 blockade/maraviroc) that have immunosuppressive qualities. Finally, opportunistic infections have not been problematic in the HIV infected recipients. HHV8 mediated Kaposi's sarcoma can be controlled with rapamycin. However, HPV mediated anal/cervical cancers may be problematic, and will require rigorous monitoring following transplantation.

I-110

*Targeting the PD-1/PD-L1 pathway to achieve a functional cure for chronic infection*

**JoAnn Suzich, PhD**, MedImmune

Like cancer, chronic viral infection is associated with non-productive T cell responses resulting in limited clearance of virus and virus-infected cells. We conducted an in vitro evaluation of virus-specific responses in peripheral blood mononuclear cells (PBMCs) isolated from subjects chronically infected with HBV or HIV, and investigated whether blockade of the PD-1/PD-L1 pathway with an anti-PD-L1 monoclonal antibody (mAb) currently under development by MedImmune could improve virus-specific T cell responses. Methods: Cryopreserved PBMCs were thawed and expression of PD-1 and PD-L1 was measured either directly ex vivo or following stimulation with virus-specific peptides. PBMCs were also tested in IFN- $\gamma$  or IL-2 ELISpot assays to monitor for HIV and HBV-specific T cell responses in the presence or absence of PD-1 or PD-L1 blocking mAbs or isotype-matched control mAbs. Results: As was reported by other investigators, PD-1 was found to be upregulated on virus-specific T cells from subjects with chronic infection. In addition, monocytes from chronically-infected subjects expressed PD-L1, and this expression was up-regulated following stimulation with virus-specific peptides. HIV and HBV-specific T cell responses were enhanced by an anti-PD-L1 agonist antibody. However, preliminary data suggests that chronic PD-1/PD-L1 blockade can result in expanded but non-functional virus-specific T cell populations associated with up-regulation of co-inhibitory receptors. Conclusion: A limited course with a PD-1/PD-L1 blocking mAb may have utility in the treatment of chronic infection and could be an important component of a functional cure.

I-111

*Closing Remarks*

**Robert Gallo, MD**, Institute of Human Virology



P-A1

*Cancers attributable to infectious agents in Nigeria: 2012-2014***Michael Odotola, MD**, Institute of Human Virology, Nigeria

Odotola M, Jedy-Agba E, Oga E, Igbinoba F, Out T, Ezeome E, Ekanem I, Hassan R, Adebamowo C. Background Infections by certain viruses, bacteria, and parasites have been identified as risk factors for some cancers. We carried out this study to evaluate the numbers of cancers in Nigeria from 2012-2014 attributable to infections using data from Population Based Cancer Registries [PBCR] in Nigeria. Methods We considered cancers associated with Epstein-Barr virus [EBV], Human Papilloma Virus [HPV], Hepatitis B and C Virus [HBV/HCV], Human Immunodeficiency Virus and Human Herpes Virus 8 [HIV/HHV8], Helicobacter pylori and Schistosoma haematobium that have been classified as oncogenic by IARC. We obtained data on infection associated cancers from the registry databases of 3 PBCR in Nigeria; Abuja, Enugu and Calabar cancer registries. We used Population Attributable Fraction for infectious agents associated cancers in developing countries that were calculated using prevalence data and relative risk estimates in previous studies. Results The 3 PBCR reported 4,861 cancer cases from 2012-2014; 1,875 in males and 2,986 in females. There were 412 infection-associated cancers in males accounting for 22% of total cancers in males, and 351 [85%] of these were attributed to infections. In female, there were 727 infection-associated cancers accounting for 24% of all cancers in females and of these, 674 [93%] were attributable to infections. Cancers of the Cervix [n=430] and Liver [n=152] as well as Non-Hodgkin's Lymphoma [n=129] were the commonest infection-associated cancers in both sexes. The commonest infectious agents associated with cancers were HPV [n=453], HIV/HHV8 [n=199], HBV/HCV [n=143] and EBV [n=125]. Conclusion Our finding suggests that 85% of infection-associated cancers in males and 93% infection-associated cancers in females can be prevented with vaccination, safer risk behaviours or anti-infective treatments.

P-A2

*Shooting Many Cytokines by a single stone.***Xiaorong Wu, BS**, Institute of Human Virology; **Bana Jabri, PhD**, University of Chicago; **Azimi Nazli, PhD**, BIONIZ Therapeutics; **Yutaka Tagaya, MD, PhD**, Institute of Human Virology

[Introduction] We are developing a new type of cytokine inhibitor that can block the action of more than 2 structurally related cytokines with selective target specificity. The in vitro proof-of-concept has been recently published (Nata et al JBC 2015, Massoud et al PNAS 2015). The PEGylated BNZ132-1 peptide strongly blocked IL-2 and/or IL-15 in vivo (in mice and macaques) but showed marginal toxicity. These two cytokines play crucial role for the activation of CD8 T cells in the development of HAM/TSP (HTLV-1 associated myelopathy). We will be commencing a clinical trial involving BNZ132-1 and HAM-TSP patients at the NIH this year. Functionally related cytokines often cause human diseases which cannot be treated efficiently by a single antibody therapy. Therapeutic combination of antibodies is too costly. Thus, our approach fills in the existing gap of the current therapy. [Discussion] We are now testing the second peptide (BNZ132-2) that blocks IL-21 and -15. These two cytokines play causative roles in inflammatory bowel diseases and in the Celiac Disease. In particular, refractory Celiac disease (RCD) that shows little response to gluten-free diet is our target. RCD also shows high association of T-cell leukemia. Using IELs from Celiac Patients, we have identified unique combinatorial effects of IL-21 and IL-15 in signaling events. Celiac Lymphocytes seem more dependent on the IL-15/21 combination. RNA-Seq also validated that some critical CTL genes are efficiently upregulated in response to the IL-21/15 combination. Most importantly, BNZ132-2 strongly blocked the IL-15/21 combination at many levels. [Conclusion] RCD is extremely difficult to treat at the moment. Our peptide BNZ132-2 would provide a promising candidate for a novel treatment for RCD. Additionally, the combined use of BNZ132-1 and -2 blocks IL-2, -15, -9 and -21, majority of the T-cell activating cytokines without affecting the homeostasis of T-cells (maintained by IL-7) and would be a useful tool to control fatal cytokine storm upon viral infections.



P-A3

*Expression of Evolutionarily Novel Genes in Tumors*

**Andrei Kozlov, PhD**, The Biomedical Center and Peter the Great St. Petersburg Polytechnic University; **Ekaterina Matyunina, Junior Researcher**, The Biomedical Center and Peter the Great St. Petersburg Polytechnic University; **Andrei Makashov, Junior Researcher**, The Biomedical Center and Peter the Great St. Petersburg Polytechnic University; **Larisa Krukovskaya, Senior Researcher**, The Biomedical Center and Peter the Great St. Petersburg Polytechnic University

The expression of evolutionarily novel genes in tumors was predicted by hypothesis of the possible evolutionary role of tumors (A.P. Kozlov, "Evolution by Tumor Neofunctionalization", Elsevier/Academic Press, 2014). In my lab we described several genes with dual specificity – evolutionarily novel genes expressed specifically or predominantly in tumors (OTP, ESRG, PVT1, ELFN1-AS1, HHLA1, DCD, SPRR1A, CLLU1, PBOV1 and others). We also described the evolutionary novelty of the whole classes of genes expressed predominantly in tumors, i.e. CT-X genes and genes of noncoding tumor specifically expressed RNAs. We studied the phylogenetic distribution of the orthologs of genes expressed in tumors and found that different functional gene classes have different evolutionary novelty. Some of them are enriched with evolutionarily novel genes. We showed that evolution of oncogenes, tumor suppressor genes and differentiation genes occurred in a parallel way, which supports the participation of tumors in the origin of new cell types. Some human genes which determine progressive traits originated in fishes and were first expressed in fish tumors. I also studied the literature data on the specificity of expression of evolutionarily novel genes originated through different molecular mechanisms. The existing data suggest that genes originated by gene duplication; from endogenous retroviruses; by exon shuffling; and de novo are expressed in tumors, sometimes with high tumor specificity. The conclusion is made that the expression of evolutionarily novel genes in tumors may be a novel biological phenomenon with important evolutionary role.

P-A4

*Distinct subcellular localization of HTLV-1 HBZ oncoprotein in the ATL leukemic and HAM/TSP patients*

**Marco Baratella, PhD**, University of Insubria; **Goutham Raval, PhD**, University of Insubria; **Greta Forlani, PhD**, University of Insubria; **Alessandra Tedeschi, MS**, University of Insubria; **Antoine Gessain, MD**, Institut Pasteur; **Giovanna Tosi, PhD**, University of Insubria; **Roberto Accolla, MD, PhD**, University of Insubria

HTLV-1 is the etiological agent of a severe form of T cell neoplasia called Adult T cell Leukemia (ATL) and of a neurologic disorder designated HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The HBZ oncoprotein encoded by the minus strand of the HTLV-1 is thought to play an important role in both diseases. Nevertheless, due to the lack of suitable reagents to assess the expression of endogenous HBZ, it has been difficult until now to strictly associate function, level of expression and sub-cellular localization to the disease status. The isolation of the first described monoclonal antibody against HBZ, 4D4-F3, in our laboratory has recently allowed to study the above parameters in cells of HTLV-1 infected patients and in ATL patients. HBZ is expressed in speckle-like structures localized in the nucleus in chronically infected cells and in ATL. HBZ interacts in vivo with p300 and JunD and co-localizes only partially not only with p300 and JunD but also with CBP and CREB2. We have extended the analysis to cells of HAM/TSP patients. Remarkably, HBZ is exclusively localized in the cytoplasm of PBMC from HAM/TSP and HBZ-positive cells do not overlap with cells expressing Tax-1, the other major HTLV-1 oncogene. Our results establish for the first time a diverse pattern of sub-cellular localization of endogenous HBZ protein. Furthermore, our results suggest that the endogenous localization of HBZ protein in different cellular compartments may correlate with the different forms of the HTLV-1-mediated diseases. On the basis of these results we propose the HBZ cytoplasmic localization as a bona fide specific biomarker of HTLV-1-derived HAM/TSP pathology. Future studies will be addressed to the assessment of cytoplasmic HBZ localization during HTLV-1 infection and in the follow-up of infected people before they acquire clear signs of pathology, to possibly identify the cytoplasmic HBZ localization not only as a biomarker but also as a predictive element of HAM/TSP development.





P-A5

*Genetic and epigenetic changes as biomarkers of progression in HPV-related cancers*

**Franco Buonaguro, MD**, Istituto Nazionale Tumori - IRCCS "Fondazione Pascale", Napoli - ITALY; **Maria Lina Tornesello, PhD**, Istituto Nazionale Tumori - IRCCS "Fondazione Pascale", Napoli - ITALY; **Luigi Buonaguro, MD**, Istituto Nazionale Tumori - IRCCS "Fondazione Pascale", Napoli - ITALY

Persistent infections of high-risk human papilloma viruses (HR-HPVs) are often associated with progression to mucosal cancers in ano-genital as well as oropharyngeal areas. Also for cervical cancers, for which screening programs are available, it is difficult to identify the lesions at high risk of progression to invasive cancers. Molecular markers able to identify viral infections associated with progressing cervical neoplasia are strongly needed for cervical cancer screening and triage. In particular predictive biomarkers are needed for detecting lesions at high risk of recurrence/progression in order to implement appropriate treatment and for avoiding overtreatment of those at high probability of regression. In order to achieve such goal we have performed the expression profile analysis of p53-related genes in HPV16-positive genital carcinomas along with autologous non-tumor tissue, and identified significant differences in the expression levels of genes involved in regulation of apoptosis, cell cycle, proliferation and DNA repair pathways. In particular, BRCA1, CDKN2A (p16), CASP2 and TNFRSF10B genes were significantly up-regulated ( $p < 0.05$ ) in cancer lesions and appear to be good candidates for predictive biomarkers. More recently analysis of TERT-gene promoter are showing the high frequency of activating mutations (30.4% of penile cancers as well as 26.1% of cervical cancer) in lesions associated with less oncogenic HPV genotypes (i.e. HPV53), and even in HPV-negative lesions. Validation of these candidate biomarkers is currently in progress on a larger number of cases, including different grades of HPV-related neoplastic lesion (CIN1-3 and invasive cervical cancer) in association with epigenetic markers (i.e. gene methylation and miRNA expression profiles). Such studies will contribute to the development of new tools for the identification of premalignant lesions at high risk of progression to invasive cervical carcinoma.

P-A6

*Expression of HIV-1 Matrix Protein p17 and Correlation with B Cell Lymphoma in HIV-1 Transgenic Mice*

**Virginia Carroll, PhD**, Institute of Human Virology, University of Maryland School of Medicine; **Mark Lafferty, PhD**, Institute of Human Virology, University of Maryland School of Medicine; **Luigi Marchionni, MD, PhD**, Department of Oncology, Johns Hopkins University School of Medicine; **Joseph Bryant, DVM**, Institute of Human Virology, University of Maryland School of Medicine; **Robert Gallo, MD**, Institute of Human Virology and Department of Medicine, University of Maryland School of Medicine; **Alfredo Garzino-Demo, PhD**, Institute of Human Virology and Department of Microbiology and Immunology, University of Maryland School of Medicine, and Department of Molecular Medicine, University of Padova, Italy

HIV-1 infection is associated with increased risk for B cell lymphomas. Since HIV does not infect B cells, alternative mechanisms for transformation have been proposed. Current hypotheses on lymphomagenesis, based on immunosuppression, activation and/or inflammation, are generic and do not provide mechanistic, testable models. We hypothesized that HIV structural proteins may contribute to lymphomagenesis directly, as they can persist long-term in lymph nodes in the absence of viral replication. The HIV-1 transgenic mouse Tg26 carries a non-infectious HIV-1 provirus lacking part of the gag-pol region, thus constituting a model to study the effects of viral products in pathogenesis. About 15% of Tg26 mice spontaneously develop leukemia/lymphoma. Of the viral proteins examined, only expression of HIV-1 matrix protein p17 correlated with leukemia/lymphoma development and was highly expressed in bone marrow prior to disease. The tumor cells resembled pro-B cells, and were CD19+IgM-IgD-CD93+CD43+CD21-CD23-VpreB+CXCR4+. Consistent with the pro-B cell stage of B cell development, microarray analyses revealed enrichment of transcripts including Rag1 and Rag2 in lymphoma cells. We confirmed RAG1 expression in Tg26 tumors and hypothesized that HIV-1 matrix protein p17 may induce RAG1 in B cells directly. Stimulation of human activated B cells with p17 enhanced RAG1 expression in 3 of 7 donors, suggesting that intracellular signaling by p17 may lead to genomic instability and transformation. Thus, in addition to the reported angiogenic and lymphangiogenic activity of p17, extracellular matrix protein p17 has the potential to induce the enzymes responsible for DNA rearrangement and recombination in B cells.



P-A7

*Novel approach to N-glycan analysis and detection of Endo H-like activity in Human Tumor Specimens*

**Mikalus Popovic, MD, PhD**, Institute of Human Virology

Post translational modification of proteins takes place via glycosylation, and changes in glycan structure, which can be associated with biological function, are seen in malignancies. Efficient assessment of glycans, particularly in clinical settings, can be hampered by specimen size, and lengthy sample preparation. We have developed an effective procedure for N-glycan analyses using chloroform-methanol (CM) extraction of specimens in the absence or presence of water (CMW), prior to enzymatic cleavage with PNGase F. This procedure was used to determine glycan profiles in cancer cell lines, and biopsies from lung cancer. Subsequently, to demonstrate the broad applicability of the method, cancer cell lines originating from other types of tumors were also studied, including non-Hodgkin B-lymphoma, choriocarcinoma and histiocytoma. The method was successfully applied to investigation of N-glycans from small numbers of in vitro cultured cells ( $\leq 1 \times 10^5$ ) and to tumor tissues, including patient biopsies of small size. MALDI-MS analysis confirmed the efficient release of all N-glycan types, including complex forms with poly-N-acetyllactosamine chains. Importantly, in patient biopsy specimens and others, the non-aqueous CM extraction yielded high-mannose glycans with one GlcNAc moiety, suggesting preservation of an Endo-H like enzyme activity. This method enables practical application of glycan profiling to small clinical specimens, as well as detection of Endo H-like enzymatic activities in cancer cells, which is a previously unrecognized phenomenon.

P-A8

*Therapeutic targeting of intracellular toll-like and interleukin-1 receptor signaling for inhibiting infection, inflammation, and cancer.*

**Greg Snyder, PhD**, Institute of Human Virology, Department of Medicine, School of Medicine, University of Maryland, Baltimore, MD.

Lindsey Brown<sup>1,2</sup>, Daniel Deredge<sup>3</sup>, Patrick Wintrobe<sup>3</sup>, Thomas Miethke<sup>4</sup>, Stefanie Vogel, Kari Ann Shirey<sup>5</sup>, Greg A. Snyder<sup>1,2,5</sup> The Toll-like and Interleukin-1/18 families of receptors mediate immune signaling in response to pathogen, cytokines and self-antigens. Dysregulation of host based Toll/IL-1 receptor (TIR) signaling is involved in numerous human pathologies involving inflammation, autoimmunity and cancer. We propose a host-based molecular approach via regulation of innate immune TIR signaling: 1) will allow for protection against multiple pathogens (bacteria and viral) at the same time; 2) Is decoupled from receptor-pathogen interactions and therefore requires no a priori knowledge of the microbe or host -ligand interaction, and 3) provides a novel and broad development platform for controlling inflammation and disease. Accordingly we have previously solved the crystal structures of several TIRs and characterized their molecular interactions with subversive microbial TIR protein mimics using, hydrogen deuterium exchange mass spectrometry (HDX-MS), X-ray crystallography and NMR. Our molecular studies of host and microbial TIR domains identify atomic interactions and druggable sites, which have lead to development of novel small molecule and peptide compounds that selectively inhibit intracellular TIR signaling pathways in vivo and in vitro. As a proof of concept for this host-focused therapeutic approach targeting of intracellular TIR signaling, preliminary data show the pre-clinically approved small molecule inhibitor TAK-242 protects WT C57BL/6 mice against ALI and lethality using the mouse-adapted strain of influenza, A/PR8/8/34 (PR8). TAK-242 targets the TIR domain of TLR4. We are now evaluating TIR inhibitors in models of cancer. Our long-term goal in this host-centered structure-function approach is regulation of acute and chronic inflammation resulting from pathogen infection, immune dysfunction and disease.



P-A9

*Molecular Studies in the HIV-1 Transgenic mouse with PCNSL lymphoma***Joseph Bryant, DVM, PhD**, Institute of Human Virology

HIV Primary central nervous system lymphoma (PCNSL) is a malignant diffuse large B cell lymphoma that occurs in 3-5 % HIV patients. It is an aggressive disease with a poor prognosis. Studies on PCNSL are difficult to do in humans. Animal models have been critical in making progress in understanding of HIV PCNSL pathogenesis and investigating potential therapeutic strategies. The biology of PCNSL at the molecular level has not been well characterized. The HIV-1 Tg26 mouse model develops PCNSL similar to what is seen in HIV PCNSL. We have evaluated the HIV1 Tg mouse model at the molecular level. The hippocampus plays crucial role in cognatic function. Memory deficits are characteristic of HIV-associated neurocognitive disorders and involved with hippocampal pathology. PCNSL associated pathology affects cognatic function. Immunohistochemical staining in the hippocampal region of PCNSL associated TG26 mice showed increased positive cells for Baff (B cell transcription factor), CD20 (B cell marker), CD3 (T cell marker), CD45 (leukocyte marker), GFAP (astrocyte marker) and CD11b (macrophage / microglial marker). Furthermore, in the hippocampus region of these mice, NAMPT (NAD(+) biosynthesizing enzyme), SIRT1 (The NAD(+)-dependent deacetylase) SIRT3 (mitochondrial deacetylase) and PGC1- $\alpha$  (a key enzyme involved in mitochondrial biogenesis and function) expressing cells were increased. We also found increased activity of nuclear factor kappa B (NFkB) p-65 along with increased PGC1- $\alpha$ , in the hippocampus of B lymphoma associated in Tg26 mice. Earlier it has been reported that the expression of NAMPT was generally high in the more aggressive malignant lymphoma (Olesen et.al 2011 APMIS 119: 296-303). The NAMPT inhibitor, APO866, is currently in clinical phase of trials in lymphomas. It has been reported that PGC1 $\alpha$  reprograms cancer cell metabolism and stimulates mitochondrial biogenesis via regulating several mitochondrial genes. See comment in PubMed Commons below Taken together our finding suggested NAMPT/SIRT1/SIRT3/PGC1 $\alpha$ /NF-KB signaling are activated in the hippocampus of PCNSL in the HIV-1 TG26 mouse model. The identification of cellular and metabolic changes in hippocampus of these mice may provide novel insight into the basic mechanisms underlying key cognatic deficit associated with PCNSL. Furthermore, our findings suggest that PCNSL plays an important role in signaling for both cellular and metabolic stress in cognatic dysfunction induced by mitochondrial dysfunction. In conclusion our study suggests in PCNSL, inhibition of NAMPT/SIRT1/SIRT3/PGC1 $\alpha$ /NF-KB pathway might represent a novel therapeutic approach.

P-B1

*Characterization of broadly neutralizing nanobodies from dromedaries immunized with soluble trimeric subtype C SOSIP proteins*

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Nanobodies or VHH are the smallest naturally occurring antibody fragments derived from heavy chain only antibodies from Camelidae. They are characterized by high stability, high affinity, and high specificity for their target antigens, as well as having extended CDR3 loops. These properties make nanobodies suitable tools for therapeutic and preventive applications. We immunized dromedaries with gp140 SOSIPs from HIV-1 subtype C, generated VHH phage libraries from these animals, and selected Env-specific VHHs on the autologous SOSIPs. After screening 1300 phage clones, we tested 80 Env-specific soluble VHHs for neutralizing activity against the autologous and a heterologous subtype B pseudovirus in the Tzm-bl assay. Eight neutralizing VHH were further tested against a panel of 21 HIV-1 pseudoviruses, which includes the 12 pseudoviruses from the extended global panel of HIV-1 reference strains for standardized assessment of vaccine-elicited neutralizing antibodies (deCamp et al., 2014). All 8 VHHs showed neutralization of Tier 2 pseudoviruses from at least two and up to six different subtypes including circulating recombinant forms (CRF). Although the neutralization breadth differed for the individual VHHs, some VHHs showed complementary neutralization patterns covering 19 of 21 pseudoviruses in our panel including the epidemiologically most relevant subtypes C and A. Preliminary epitope mapping data by competitive ELISAs with known bnAbs, as well as negative stain EM structures with trimeric SOSIPs, identified the CD4 binding site as the major target. The broadly neutralizing nanobodies identified here are promising candidates for further development as prophylactic/therapeutic treatments of HIV-1 infection.



P-B2

*HIV Envelope gp120 fused to IgG1 Fc fragment exhibits augmented immunogenicity compared to unmodified immunogen and elicits a neutralizing antibody response in rhesus macaques*

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A major focus of HIV prevention programs is the development of a safe and effective vaccine. A successful vaccine will elicit broadly neutralizing antibodies (bNAbs) with high durability and protect against both sexual and blood-borne HIV transmission. Most neutralizing antibodies target critical epitopes on the surface, or gp120, portion of HIV envelope glycoprotein (Env). We developed a new immunogen by engineering a fusion protein containing HIV gp120 (BaL strain) with a Gly/Ser linker fused to each arm of the Fc domain of rhesus macaque IgG1 (Env-rFc). We envisioned that Env-rFc, which mimics the behavior of immune complexes, would bind to Fc gamma receptors on antigen-presenting cells to increase the strength, breadth and durability of Env-specific antibody responses. The Env portion retained structure and function, as it was capable of binding to cell surface CD4. The Fc portion was also functional, demonstrating direct binding to Fc gamma receptor followed by rapid cell uptake and accumulation in cytoplasmic vesicles. We then conducted an immunization study in rhesus macaques, comparing Env-rFc and Env (gp120 monomer) delivered by the intramuscular route. Overall, Env-rFc proved superior to Env monomer. Env-rFc elicited higher titer antibodies with increased breadth capable of recognizing CD4-induced epitopes. Env-rFc also elicited antibodies capable of neutralizing Tier1 HIV pseudotyped viruses and the serum antibodies were more durable compared to serum IgG responses seen with Env monomer immunization. The clear differences between Env monomer and Env-rFc indicate a strong advantage of the fusion protein strategy that may be improved by dose and adjuvant optimization.

P-C1

*Preservation of lymphopoietic potential and virus suppressive capacity by CD8+ T-cells in HIV-2 infected controllers*

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HIV-2 infection represents a unique model of attenuated infection by a human immunodeficiency virus. Compared to HIV-1 infection, HIV-2 infection is characterized by slower CD4+ T cell count decline and disease progression in the absence of antiretroviral therapy. Although HIV-2 infected individuals are usually characterized by low viral load and immune activation levels, the underlying mechanisms remain poorly understood. A better understanding of HIV-2 pathogenesis is central to open new therapeutic avenues to establish natural control of HIV-1 replication in infected patients. Here, we studied the capacity of HIV-2 infected patients to support long term renewal of the CD8+ T cell lymphocyte compartment by preserving immune resources, including lymphoid progenitors and thymic activity, and the efficacy of effector CD8+ T cells to control the virus. Our findings suggest that preservation of the host immune resources supports the maintenance of a strong CD8+ T cell capacity to suppress HIV-2. This effective and durable antiviral response likely participates to establishing a virtuous circle, during which controlled viral replication permits the preservation of potent immune functions, and vice versa, thus preventing disease progression in HIV-2 infected patients. Owing to the maintenance of a strong lymphopoietic capacity in infected patients, T cell mediated immune responses appears to be better preserved in HIV-2 infection and can sustain a very effective suppression of viral replication over several years of chronic infection. HIV-2 specific CD8+ T cells display an early or young differentiated phenotype, potentially reflecting T cell renewal potential. Next, they harbor particularly potent effector functions, highlighted by their exceptional capacity to suppress the virus in autologous CD4+ T cells. These CD8+ T cell characteristics are similar to those observed in HIV-1 infected elite controllers in many aspects, and are likely to be both the cause and the consequence of this effective control of the virus.





P-C2

*Characterization of the Early Antibody Landscape In HIV-1 Infected Individuals Who Develop Poor to Elite Levels of Neutralization Breadth*

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Coevolution of HIV-1 envelope (Env) glycoproteins with the host antibody (Ab) response is a complex, dynamic process. Both are subject to high levels of mutation, either via error-prone reverse transcriptase in Env or somatic hypermutation (SHM) in Ab. And, while Env is subject to selection by neutralizing Ab (nAb), Env-specific B cells must compete with one another for antigen binding and survival signals. This evolutionary race results in the development of broadly neutralizing antibody (bnAb) activity in some patients, but not others. The coevolutionary path between Env and an autologous bnAb lineage has been explored in a few individuals. We have performed a more comprehensive characterization of the early anti-Env Ab landscape in individuals with varied levels of bnAb, on the premise that these responses are more comparable to Abs that can be elicited by vaccination. We have recovered ~200 anti-Env-gp120 monoclonal antibodies (mAbs) from 6 individuals enrolled in IAVI Protocol C ~7 months post-infection. These individuals display a range of neutralization breadth ~3 years post-infection, from poor to elite. mAbs were characterized by analyzing the DNA sequences of immunoglobulin heavy (VH) and light (VL) chain variable domains, ability to neutralize autologous transmitted/founder (T/F) Env, and binding affinity (KD) for the T/F gp120. Each individual exhibited a unique VH and VL germline landscape. Induction of bnAb-associated germplines, a high level of SHM, or long CDR-H3s was not predictive of bnAb development. Very high affinity early mAbs were associated with poor nAb breadth. Our studies reveal that premature induction of a clonal, VH-biased Ab response, with early acquisition of high KD, could be detrimental to the development of bnAb. Instead, induction and maturation of a bnAb lineage may initially require balanced VH germline usage, followed by substantial interplay between Env and Ab, to steadily increase affinity and drive acquisition of breadth.

P-C3

*Analysis of the immunosuppressive properties of a trimeric recombinant transmembrane envelope protein gp41 of HIV-1*

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HIV-1 induces like many other retroviruses a severe immunodeficiency in the infected host. Inactivated retroviruses, their transmembrane envelope (TM) proteins and synthetic peptides corresponding to a highly conserved domain in their TM protein, the so-called immunosuppressive (Isu) domain, have been shown to induce inhibition of lymphocyte proliferation, as well as a modulation of cytokine release and expression of cellular genes. Therefore it was suggested that the Isu domain of the retroviral TM proteins is involved in immunopathogenesis. Point mutations in the Isu domain have been shown to abrogate the biological activity. To study this in more detail, a recombinant gp41 was produced in a human cell line. The protein was purified and characterised by several methods showing glycosylation of the protein and assembling into trimers. Binding studies by ELISA and surface plasmon resonance using conformation-specific monoclonal antibodies imply a six-helix bundle conformation. Purified gp41 bound to monocytes and to a lesser extend to lymphocytes. In parallel, homopolymers of peptides corresponding to the Isu domain were also found to bind specifically to monocytes and B cells. Gp41 triggered the production of specific cytokines when incubated with human peripheral blood mononuclear cells. Among them IL-10. Furthermore, gp41 expressed on murine target cells inhibited the antigen-specific response of murine CD8+ T cells by impairing their IFN $\gamma$  production. The expression of CD25 was also impaired. These data confirm previous findings on the interspecies-reactivity of the Isu domain and suggests highly conserved binding proteins. In summary, these data provide additional evidence that gp41 might be directly involved in HIV-1 immunopathogenesis.



P-C4

*The MHC class II transactivator CIITA acts as a restriction factor for HIV in human myeloid cells*

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We previously found that the MHC-II transactivator CIITA inhibits HIV-1 replication in human T cells by competing with the viral transactivator Tat for the binding to Cyclin T1 of Positive Transcription Elongation Factor b (PTEF-b). In this study we analyzed the anti-viral function of CIITA in myeloid cells, the other relevant HIV target cell type. We used the U937 promonocytic Plus and Minus clones, characterized by efficient or inefficient capacity to support HIV-1 replication, respectively. This different phenotype has been also associated with the viral restriction action of TRIM22 because this factor is expressed in U937 Minus but not in Plus cells. We demonstrate that CIITA is expressed only in Minus cells and as a consequence of this, Tat-dependent HIV-1 LTR transactivation is inhibited. Indeed, the stable expression of CIITA in Plus cells inhibited Tat-mediated activation of the viral LTR and reduced HIV-1 replication with respect to Plus parental cells. This result was independent from TRIM22 because CIITA did not induce TRIM22 expression in Plus transfected with CIITA. Thus, CIITA acts as a viral restriction factor against HIV-1 not only in T cells but also in monocytes. Nevertheless, Tat transcriptional activity and HIV-1 replication were inhibited to a lesser extent in Plus cells transfected with CIITA with respect to Minus cells, suggesting that CIITA and TRIM22 might still work in concert to counteract HIV-1 infection and spreading. These results will be discussed in the context of the present knowledge of cell permissivity to HIV-1 replication and to the possible involvement of specific these restriction factors in the generation and maintenance of HIV-1 silent reservoirs.

P-C5

*NKG2C+ CD8 NK-T cells – bridging the innate and adaptive immunity*

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[Introduction] We study a special subset of CD8 T cells that expresses NK-receptors which we discovered in the past. They (CD8 NK-T cells) can be activated in two ways - through TCRs and through NK receptors. Therefore, CD8 NK-T cells bridge the innate and adaptive arms of the immune response. Infectious viruses employ various strategies to evade killing actions by NK and CD8 T cells. However, CD8 NK-T cells seem impervious to typical interference by viruses and can be exploited as a front line defense against chronic virus infections. [Discussion] NKG2C belongs to the lectin family and transduces a true activating signal, unlike NKG2D which only provides a co-stimulatory signal. NKG2C+CD8 NK-T cells is a minor subset (1~5 % in CD8 T cells), and do not express typical inhibitory receptors (NKG2A, KIR2L1, LAG3, 2B4, Tim3, or CTLA4) so not easily suppressed by regular mechanisms once activated. Engagement of NKG2C specifically augmented cytotoxicity of these cells against target cells independent of specific antigen. In parallel, typical T cell activation (PHA, PMA, anti-CD3/CD28) also augmented a potent cytotoxicity. Curiously, antigen-stimulation caused a transient upregulation of PD-1, a checkpoint molecule, which terminated the TCR-mediated killing of these cells in the long run. However, the NKG2C-induced cytotoxicity was not affected by the PD-1 engagement. Thus NKG2C+ CD8 NK-T cells uses the PD-1 system as a molecular switch to choose between the innate and adaptive modes of target killing. [Conclusion] Despite its unique nature, NKG2C CD8 NK-T cells have not been well-characterized. Our study provides a potential of these cells as a versatile killer which operates in the innate and adaptive modes and are more resistant to the immune evasion by viruses. Previous studies show their role in the CMV infection. Ex vivo expansion and arming with self-activating HLA-E molecule of these cells may help develop a potent cellular defense against persistent infections including that by HIV.



P-C6

*Tyrosine-Sulfated Peptides from the V2 Loop of gp120 Are Potent HIV-1 Inhibitors*

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The second variable (V2) loop of HIV-1 gp120 is the focus of increasing attention both as a functional domain and as a vaccine target. Recently, we identified two tyrosine sulfation sites in this region that may help to stabilize the envelope trimer in a native state through intramolecular interaction with V3 base. To further investigate the functional role of this region and its potential usefulness as a template for the design of new HIV-1 inhibitors, we created tyrosine-sulfated peptides based on the V2 sequence and showed that they efficiently bind to both monomeric and trimeric gp120 in a CD4-dependent manner. Furthermore, tyrosine-sulfated V2 peptides, but not their unsulfated counterpart, potently inhibit HIV-1 entry and fusion by preventing coreceptor utilization, with the highly conserved C-terminal sulfotyrosine, Tys177, playing a dominant role. Unlike CCR5 N-terminus-derived sulfated peptides, we found that V2 peptides inhibit a broad range of HIV-1 isolates irrespective of their coreceptor tropism. Due to their potency, specificity and breadth of antiviral activity, sulfated V2 peptides may represent promising candidates for the design of new HIV-1 entry inhibitors.

P-C7

*Differential host gene expression in HIV-1 and HIV-2 infected monocyte derived macrophages (MDM)*

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Disease progression due to HIV-1 and HIV-2 infection differs at the patient level and is dependent on the host transcriptional machinery. Understanding the variations of cellular responses between infection with HIV-1 and HIV-2 could reveal important insights into their pathogenesis and provide new tools that could serve as prognostic markers and targets for therapeutic intervention. To achieve this, we analyzed the effects of HIV-1 (BaL) and HIV-2 (ROD) infection on the expression of host factors in MDMs at the RNA level using the Agilent Whole Human Genome Oligo Microarray platform. Host gene expression was analyzed using the GeneSpring software. Differentially expressed genes were identified and their biological functions determined. The top fifty genes were validated by RT-PCR, using RNA isolated from MDM infected with either HIV-1 or HIV-2. Host gene expression profiles were significantly different at 7 days post infection compared to non-infected cells. Genes involved in glutathione metabolism, lysine degradation, olfactory transduction, nicotinate and nicotinamide metabolism were differentially expressed. Differences in the expression pattern of non-coding RNA and olfactory receptors were also observed. Host genes SRSF9, PIKFYVE, CUL-2 and DDX3X were upregulated only in HIV-2 infected cells, while ZNF568 and RNF157 genes were down regulated only in HIV-1 infected cells. These studies provide additional insight into the mechanisms underlying the delayed disease progression observed in HIV-2 infected individuals and help to predict severity of disease progression due to infection with the two types of HIV strains.



P-C8

*Directly Acting Antiviral Therapies have differential effects on cellular and soluble markers of inflammation in successfully-treated HIV/HCV co-infected patients*

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Background: HIV/HCV co-infected patients have higher levels of immune activation and accelerated fibrogenesis. We compared markers of immune activation in HIV/HCV co-infected subjects successfully treated for HCV with one of three DAA-only regimens in order to determine if different agents have different effects. Methods: Two prospective phase II studies conducted at the NIH Clinical Center studied DAA-only regimens (SOF/LDV for 12 weeks in ERADICATE (NCT01878799) and DCV/ASV, two drugs for 24 weeks, or with beclabuvir for 12 weeks in CONQUER (NCT02124044)) in HIV/HCV genotype 1 co-infected subjects. We tested paired pre/post-treatment samples from a subset of patients via a multiplex assay (ProcartaPlex Human Cytokine & Chemokine Panel, eBioscience) and flow cytometry for markers of activation (CD38, HLA-DR) and exhaustion (TIM-3, PD-1, CTLA-4). Median values are reported; comparisons were made using Wilcoxon Rank tests. To correct for multiple comparisons,  $P < 0.02$  was considered significant. Results: Subjects from ERADICATE (2E, n=6), CONQUER 2DAA arm (2C, n=7) and CONQUER 3DAA arm (3C, n=7) were comparable in terms of sex, age, race, fibrosis, and CD4 counts. 2E subjects were HCV treatment naïve; 20% of 2C subjects and 58% of 3C subjects were treatment-experienced. All CONQUER patients were on ART, compared to 67% of included 2E subjects. All groups had a significant decline in the percentage of circulating activated T cells, while 3C patients showed a significant decline in chemokines and pro-inflammatory cytokines, particularly IL-10, IL-1R, IL-8, IP10, and MIP-1 beta. Conclusions: In this study, SVR is associated with reduced cellular activation, resulting in reduced cytokine production. The addition of NS3 inhibitor appears to improve resolution of cellular and soluble markers of immune activation.

P-C9

*Differentiation of MonoMac-1 Cell Line Induced by M-CSF and Glucocorticoid Pathways*

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Monocytes and macrophages have an important role in HIV-1 infection, capable of serving as reservoirs of HIV infection, but also for their potential role in modulating adaptive immunity, thereby having the potential to contribute to the pathogenesis of AIDS. Virus-host interactions appear to result in altered immune polarization and immune suppression through the process of altered macrophage immune polarization. CD163+/CD16+ monocytes are considered a more mature monocyte, and their frequency has been correlated directly with viral load and inversely with CD4+ T cell count. These cells be a precursor to alternatively activated or Type 2 macrophages, which may dampen immune responses in tissues in HIV infection. The CD163+/CD16+ monocyte subset has been reported to be preferentially infected by HIV and furthermore, carries HIV DNA in HIV infected patients. To develop strategies to reduce or target this subset therapeutically, we performed studies to establish as cell culture model system to test compounds of interest. In our studies presented here, we investigated the effects of PMA, LPS, DEX and M-CSF on the expression of CD163 and CD16 as determined by flow cytometry. Using a stepwise approach, using MONO-MAC-1 cells treated with PMA and PMA+LPS for 3 days followed by DEX+MCSF for 4 days, we were able to increase expression of CD163 and CD16. These affects appear to be due to the action of Dexamethasone and M-CSF on the cognate glucocorticoid and cFMS receptors respectively as RU486 and PLX3397 exhibited inhibitory effects. Our results may provide insights regarding the role of M-CSF and glucocorticoids on myeloid differentiation and furthermore, may emphasize the potential of targeting these pathways for therapeutic intervention in disease states such as HIV infection, cardiovascular disease, and metabolic syndrome, where this monocyte subset is increase. The culture conditions we established useful for screening other compounds that have therapeutic potential in affecting monocyte macrophage differentiation.



P-C10

*HIV, Hepatitis C, Hepatitis B Co- Infections and Their Genotypes Among Fishermen Attending Homabay District Hospital*

**Allan O. Onyango, MS, NIH**

Human immunodeficiency Virus (HIV), Hepatitis B virus (HBV), and Hepatitis C virus (HCV) are the three most common chronic viral infections all over the world. Despite their biological differences, these viruses share similar transmission routes, including sexual, blood contact, and injecting drug use. HCV, HBV, and HIV infections are one of the most important public health problems today. However, there is limited information on their prevalence, distribution and clinical significance in Kenya, where the prevalence of HBV-HCV and HIV co-infections have not been well established. The reason is that there has not been an established gold standard diagnostic system. Poverty, inadequate adequate medical services and facilities, poor quality of education services, cultural or social barriers and political turmoils are but a few of the many factors that do facilitate the long-term spread of these co-infections. The objective of the study is to determine the prevalence of HBV-HCV and HIV co-infections among fishermen patients attending Homabay District Hospital. This is a laboratory based study. Patient samples are to be obtained from fishermen patients attending Homabay district Hospital, which is a referral hospital in Homabay County for nearby clinics in its environs and tested for HIV, HBV and HCV; archived samples from those stored at the Hospital are also to be analyzed. The genotypes are to be analyzed using a polymerase chain reaction machine. The results will provide information on the Hepatitis B, Hepatitis C and HIV situation in the region, assist the decision makers to develop strategies for improvement, identify and disseminate good practices between clinics the region. The study will also help to interpret results; identify groups at risk of HBV-HCV and HIV infections suggest further research to assist in the improvement of HCV control program at the facility; provide results of the study to the management and staff at the facility. In this study, the collected data is to be analyzed using SPSS Version 22 software.

P-C11

*Adjuvant-dependent innate and adaptive immunosignatures of risk of SIVmac251 acquisition*

**Genoveffa Franchini, MD, NIH**

**Abstract** A recombinant vaccine containing Aventis Pasteur's canarypox vector (ALVAC)-HIV and gp120 alum decreased the risk of HIV acquisition in the RV144 vaccine trial. The substitution of alum with the more immunogenic MF59 adjuvant is under consideration for the next efficacy human trial. We found here that an ALVAC-simian immunodeficiency virus (SIV) and gp120 alum (ALVAC-SIV + gp120) equivalent vaccine, but not an ALVAC-SIV + gp120 MF59 vaccine, was efficacious in delaying the onset of SIVmac251 in rhesus macaques, despite the higher immunogenicity of the latter adjuvant. Vaccine efficacy was associated with alum-induced, but not with MF59-induced, envelope (Env)-dependent mucosal innate lymphoid cells (ILCs) that produce interleukin (IL)-17, as well as with mucosal IgG to the gp120 variable region 2 (V2) and the expression of 12 genes, ten of which are part of the RAS pathway. The association between RAS activation and vaccine efficacy was also observed in an independent efficacious SIV-vaccine approach. We tested adjuvants that may be able to increase the "protective profile" and found one adjuvant that significantly decreased the frequency of a4B7 Ki67+CD4+ T-cell targets of viral infection. Results of challenge exposure experiment will be presented.



P-C12

*Hepatic and Peripheral Immunophenotypic and functional differences of CCR5+ and CCR5- T-cells in HIV/HCV coinfecting and HCV mono-infected patients*

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Liver fibrosis is accelerated in HIV/HCV coinfection. CC chemokine receptor 5 (CCR5) is expressed on T-cells and plays a role in migration and activation of cells. A profibrogenic role has been described in the pathogenesis of cirrhosis. Aim: Determine effect of HIV coinfection on CCR5+ T-cell function and migration. Method: Paired peripheral blood mononuclear cells and liver infiltrating lymphocytes from 21 HIV/HCV and 14 HCV subjects were analyzed for CD4 and CD8 T-cell CCR5 expression by flow cytometry. PBMCs from 5 HIV/HCV and 5 HCV subjects were flow sorted for CCR5+ and CCR5- T-cells. Phenotypic and functional characterization by 12-color flow cytometric analysis was conducted pre and post-stimulation with HCV genotype specific overlapping pooled peptides. Statistical analysis: t-test, significance at  $p < 0.05$ . Results: Hepatic CD4 and 8 CCR5+ T-cell populations were higher than peripheral in both HIV/HCV and HCV subjects ( $p < 0.0001$ ) and higher in HIV/HCV compared to HCV ( $p < 0.0001$ ). Contrastingly, peripheral CD4+CCR5+ populations were smaller in HIV/HCV compared to HCV ( $p = 0.006$ ), but expressed more CXCR3 than HIV/HCV CD4+CCR5- ( $p = 0.04$ ) and HCV CD4+CCR5+ T-cells ( $p = 0.05$ ). Furthermore, in HIV/HCV, CD4+CCR5+ T-cells expressed more activation (HLADR+CD38+) and exhaustion (PD-1) markers (both  $p = 0.04$ ), and produced less IFN-gamma in response to HCV peptide stimulation ( $p = 0.05$ ) than CD4+CCR5+ T-cells from HCV subjects. CD8+CCR5+ T-cells from HIV/HCV subjects also expressed higher exhaustion markers Tim-3 and CTLA-4 ( $p = 0.02$  and  $0.04$ ) than HCV subjects. Conclusion: Our results suggest that in HIV/HCV coinfection, activated CD4+CCR5+CXCR3+ T-cells migrate to the liver. These cells are not HCV specific and may lead to non-specific inflammation and accelerated fibrogenesis in HIV/HCV coinfecting patients. Strategies to block CCR5 could be used as a therapeutic tool for fibrosis progression.

P-C13

*Impact of HIV status, HCV genotype, and Number of DAAs on HCV Viral Kinetics in Patients with HCV Genotype 1 Receiving DAA Therapy*

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Background: A better understanding of the impact on HCV viral kinetics (VK) of HIV status, HCV genotype, and number of DAAs used may allow further optimization of DAA regimens. Methods: VK data was evaluated for studies of patients with HCV genotype 1 mono-infection treated with ledipasvir (LDV)/ sofosbuvir (SOF) [SYNERGY A], LDV/SOF + GS-5885 [SYNERGY B], or LDV/SOF + GS-9451 [SYNERGY C]; and patients with HIV/HCV genotype 1 co-infection treated with LDV/SOF [ERADICATE], or asunaprevir (ASV) and daclatasvir (DCV) +/- beclabuvir (BCV) [CONQUER]. Viral kinetic modeling with a multiscale model was performed for all participants. Results: There was no statistically significant difference in the clearance rate of free virus ( $c$ ), treatment effect ( $\epsilon$ ), and loss of infected hepatocytes ( $\delta$ ) when comparing HCV mono-infected and HIV/HCV co-infected patients treated with regimens containing 2 DAA, when comparing HCV patients to HIV/HCV patients treated with 3 DAA, or when comparing HIV/HCV patients treated with 2 DAA regimens vs. 3 DAA regimens. However, when comparing genotype 1a and genotype 1b infection in HIV/HCV co-infected patients treated with 2 DAA or 3 DAA regimens, a favorable treatment effect ( $\epsilon$ ) was identified in those with genotype 1a ( $p < 0.001$ ). Conclusions: HIV coinfection does not seem to affect HCV viral kinetics, while genotype 1a seems to have a rapid decline of HCV kinetics with multiple DAA therapy. HCV viral kinetics could be useful in individualized, response guided therapy in identifying patients who are likely to respond to a shorter duration of therapy.

P-C14

*Association of anti-Vacc-C5 antibody titre with immune function and immune activation in HIV-1 infected subjects*

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Dysfunctional activation of the immune system is a defining feature of progressive HIV disease. It is characterised by inflammation, activated T cells and myeloid cells and the translocation of microbial products across the gut mucosa. Immune activation is present to some extent even in patients for whom the virus has been successfully suppressed through combined antiretroviral therapy (cART) and immune reconstitution is often incomplete. This residual immune activation may contribute to HIV-1 comorbidities including atherothrombosis, neurocognitive disorders and osteoporosis. Thus a greater understanding of the causes of immune activation are required in order to develop new therapies to improve immune system function and complement both cART and therapies developed from 'cure' research. We have previously identified that antibodies specific for a heterodimeric peptide construct comprising the C5501-512 region and a compatible region on gp41732-744 within the HIV envelope protein (Vacc-C5) are correlated with slow disease progression in HIV-1 infected subjects and in certain patients with a natural viral suppressor phenotype. In this study we sought to determine whether levels of anti-Vacc-C5 correlated with markers of immune function or immune activation. 45 HIV-1 infected subjects undertaking ART were recruited for the study. HIV-1 infected subjects demonstrated a range of anti-Vacc-C5 antibody titres between 0.04-19.87µg/ml. Anti-Vacc-C5 antibody titres greater than 1µg/ml were positively associated with greater CD4+ counts and increased IFN-γ responses to class I or class II restricted peptides and peptides derived from HIV-1 p24. High anti-Vacc-C5 antibody titres were inversely correlated with T cell activation measured by HLA-DR and CD38, and sera levels of IL-6. Associations with LPS, sCD14 and myeloid cell and NK cell phenotype have also been made. These data support a role of anti-Vacc-C5 antibodies in the maintenance of functional immune phenotypes in HIV-1 infected individuals.

P-C15

*Characterization and Development of a Small Novel Molecule for AIDS-NHL*

**Joseph Bryant, DVM**,

The risk for AIDS defining cancers including AIDS-NHL has declined dramatically after the introduction of combined antiretroviral therapy (cART), however, the occurrence of lymphomas in the HIV setting has not decreased to the same extent. In fact, lymphomas still represent the most common type of cancer in HIV individuals, comprising more than 50% of all AIDS defining cancers. These lymphomas have high grade features, and usually present at an advanced stage, often with extranodal involvement to include the gastrointestinal tract, CNS system, bone marrow and liver. To tackle the challenge of treating AIDS/NHL, it is crucial to discover and develop more effective and less toxic anticancer agents. To this end my lab has focused on drug discovery from natural plants. We recently isolated a small molecule that we have shown to be very potent against several cancers, one of which is AIDS/MHL. The molecule has been identified as a flavonoid (HLBT-100). Flavonoids are a most important group of polyphenols that have been demonstrated to act on multiple key elements in signal transduction pathways related to cellular proliferation, differentiation, cell cycle progression, apoptosis, inflammation and angiogenesis. The newly isolated molecule HLBT-100 has been shown to act on AIDS/NHL through the multiple pathways. We demonstrate this both in-vitro on several NHL cell lines and in-vivo in an animal model, the HIV-1 transgenic mouse model.



P-C16

*Differential Induction of Anti-V3 Crown Antibodies with Cradle and Ladle-Binding Modes in Response to HIV-1 Envelope Vaccination*

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The V3 loop in the HIV envelope gp120 is one of the immunogenic sites targeted by virus-neutralizing Abs. The V3 crown in particular has conserved structural elements recognized by cross-reactive Abs, implicating its potential contribution in protection against HIV. Crystallographic analyses of anti-V3 crown mAbs in complex with the V3 peptides have revealed that these mAbs recognize the conserved sites on the V3 crown via two distinct strategies: a cradle-binding mode (V3C) and a ladle-binding (V3L) mode. However, almost all of the anti-V3 crown mAbs studied in the past were isolated from chronically HIV-infected individuals. The extents to which the two types of anti-V3 crown Abs are generated by HIV envelope vaccines are unknown. This study analyzed the prevalence and levels of V3C-type and V3L-type Ab responses in HIV-infected individuals and in HIV envelope-immunized humans and animals using peptide mimotopes that distinguish the two V3 Ab types. The results show that both V3L-type and V3C-type Abs were generated by the vast majority of chronically HIV-infected humans, although the V3L-type were more prevalent. In contrast, only one type of V3 Abs was elicited in humans or animal models after receiving the HIV envelope vaccines. Irrespective of the HIV envelopes and immunization regimens used, the V3C-type Abs were produced by vaccinated humans, macaques, and rabbits, whereas the V3L-type Abs were made by mice. The V3C-type and V3L-type Abs generated by the HIV envelope vaccines were able to mediate virus neutralization. These data indicate the restricted repertoires and the species-specific differences in the functional V3 Ab responses induced by the HIV envelope vaccines. The study implicates the need for improving immunogen designs and vaccination strategies to broaden the diversity of Abs and target the different conserved epitopes in the V3 loop and in the HIV envelope as a whole.

P-C17

*Critical Role of V2 Sulfotyrosines in Stabilizing the HIV-1 Envelope Trimer in Its Closed, Antibody-Protected Conformation*

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Immune evasion is a hallmark of the HIV-1 envelope and represents a major barrier to vaccine development. We recently discovered that two conserved tyrosines (Y173, Y177) in the V2 loop of the gp120 envelope glycoprotein can be post-translationally modified by O-sulfation and functionally mimic the sulfotyrosines present in the N-terminal region of CCR5, stabilizing the intramolecular interaction between V2 and V31. To gain further insight into the functional role of the V2 sulfotyrosines, we examined the effects of tyrosine sulfation modulation and mutagenesis on HIV-1 neutralization sensitivity. Inhibition of tyrosine sulfation increased HIV-1 sensitivity to soluble CD4 and poorly/non-neutralizing mAbs; at the same time, neutralization by trimer-specific mAbs was reduced, suggesting that tyrosine sulfation contributes to stabilizing the closed trimer conformation. Reciprocal results were obtained upon enhancement of tyrosine sulfation. An even more dramatic effect was observed upon phenylalanine or alanine substitution of the V2 tyrosines, indicating that the tyrosine side-chains play a stabilizing role irrespective of their sulfation status. Strikingly, the V2 tyrosine mutants became highly susceptible to neutralization by HIV-1-infected patient sera, including those with weak/restricted neutralizing capacity, suggesting that the bulk of host-produced antibodies cannot reach through the tight protective shield of the native trimer. Altogether, these results document the key role played by the V2 tyrosines, particularly in their sulfated form, as a mechanism of HIV-1 immune evasion.





P-C18

*HIV-1 Env Adopts an Open Trimer Conformation in Response to Fusion Inhibitor Resistance*

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Peptide entry inhibitors block HIV entry by inhibiting membrane fusion with the target host cell. They are made up of short peptide analogs of either the N- or C-terminal heptad repeats (HRs) of gp41. These peptides act by direct binding to a gp41 fusion intermediate and preventing formation of the 6-helix bundle that drives virus-host cell membrane fusion. Previously, our group reported on two genetically distinct pathways of N-terminal peptide resistance mutation, defined by founding mutations in either the N- or C-terminal HRs – pathway 1 and pathway 2, respectively. While peptide resistance maps to gp41 mutations, both pathways include non-overlapping sets of secondary mutations in gp120, suggesting distinct routes of crosstalk between gp41 and gp120. Using a lentiviral pseudovirus system, we have evaluated the infectivity, sensitivity to nAbs, sCD4, and temperature sensitivity of Envs from both fusion inhibitor resistance pathways. Envs from both pathways adopt an ‘open’ trimer conformation, becoming more sensitive to sCD4, weakly neutralizing and non-neutralizing antibodies, while becoming more resistant to a subset of bNAbs preferring the native/closed trimer conformation. In most cases, changes in neutralization sensitivity map to Gp120 mutations. Our analysis reveals the two pathways are not equivalent, involving distinct sets of changes in both Gp120 and Gp41. Pathway 1 Envs are more sensitive to sCD4 than WT and Pathway 2 Envs, and exhibit greater neutralization sensitivity in general, suggesting a more open conformation or greater degree of conformational flexibility. Efforts are underway to understand the mechanism of these changes in the context of viral entry.

P-C19

*The in vivo effects of Anti-a4b7 in rhesus macaques during acute and chronic infection*

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ART dramatically reduces the viral burden in HIV-1 infected individuals. However, because it fails to eradicate recrudescing viremia, life-time administration, which is associated with toxicity, is required. Our hypothesis has been that protecting the gastro-intestinal tract (GIT) in HIV-1 infected individuals could alleviate the requirement for prolonged ART. We have successfully shown that the I.V infusion of a novel primatized recombinant mAb directed against integrin a4b7 just prior to and during acute SIV infection leads to a marked reduction in GIT viral loads in groups of rhesus macaques infected either intravenously or intrarectally. More recently, using a repeated low dose intra-vaginal challenge model, we determined that administration of anti-a4b7 mAb prevented SIV transmission in 50% of challenged animals. In the remaining 50% that did become infected levels of viral DNA in GIT we found to be low or undetectable despite significant viremia. Of note, protection of GIT was associated with the reconstitution of CD4 levels in both blood and gut tissues, and a clinically healthy status for > 2 years following infection. The discordance between viral DNA in GIT and viremia suggested that virus replication in these “disease protected” animals must occur in tissues other than GIT. To identify the source of viremia we developed an in vivo localization technique that utilizes PET/CT scanning of animals administered a 64Cu-labeled anti-SIV gp120 mAb as a probe. Initial analysis of PET/CT imaging data from anti-a4b7 mAb treated and infected animals suggests that such treatment leads to marked differences in the tissues in which virus replicates, with the notable sparing of the large intestine. We conclude that protecting distinct tissues, particularly those within the GIT, may be one approach to limit the progression of disease in HIV infected patients.



P-C20

*Th17, CCR6+ cells lack RNAses and are highly permissive to HIV infection: implications for pathogenesis and therapy.*

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Th17 cells (i.e., IL17-producing T cells) are selectively depleted in acute HIV infection and in pathogenic, but not in non-pathogenic, SIV infection. Since IL17 stimulates the production of antimicrobial peptides including defensins and contributes to the preservation of mucosal integrity, Th17 cell loss initiates a vicious cycle of increased mucosal permeability, microbial translocation, and inflammation that further fuels HIV infection and immune activation, contributing to HIV pathogenesis. Our studies demonstrate that Th17 cells are more permissive to HIV infection than non-IL17 secreting cells. Compared to activated, IFN- $\gamma$  secreting T cells, Th17 cells are more likely to get infected and express higher levels of HIV p24, even when HIV pseudotyped with AMLV envelope is used to infect cells, indicating that the increase in infection is not due to variations in HIV receptor expression. CCR6, a chemokine receptor expressed on Th17, cells further defines a subset of activated cells that is highly susceptible to HIV infection. Using RNA microarray technology, we determined that Th0 CCR6- cells express higher levels of RNASEs 2, 3 and 6 than Th17 CCR6+ cells. We also found that these RNASEs were more effective in inhibiting HIV in Th17 than Th0 cells, indicating that they may play a key role in reducing infection in Th0 cells. These findings point at future directions for prevention and treatment of HIV infection. First, since we characterized that beta-defensin 2 inhibits HIV infection in CCR6+ cells by inducing expression of APOBEC3G, the defensin itself or small molecule derivatives could be used to prevent loss of Th17 cells that express CCR6. Second, the elucidation of the mechanism of activity of RNASEs could help protecting highly permissive TH17 cells. Third, pathways leading to T cell activation and Th17 differentiation could be targeted to decrease cells permissivity and lower immune activation.

P-C21

*Molecular Mimicry of a Helical Region in Domain 1 of CD4 Facilitates Interdomain Stabilization of HIV-1 gp120*

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The HIV-1 envelope spike displayed on the surface of infectious virions is a homotrimer of gp120:gp41 heterodimers. In its closed, pre-fusion form, the envelope trimer features a highly flexible and metastable conformation, which facilitates immune evasion, but it undergoes dramatic conformational changes upon binding to CD4 resulting in a stable, low-energy post-fusion state. High-resolution structures of a soluble, disulfide-stabilized gp140 trimer (SOSIP) have provided important insights into the anatomy of the pre-fusion envelope. Here, we focused on a previously reported motif of 5 identical amino acids (SLWDQ) present both in CD4 (domain 1, DE loop) and in gp120 (inner domain,  $\alpha$ 1-helix), which also displays a remarkable structural homology being a right-handed helical structure in both proteins. Since the SLWDQ region of gp120 interacts intramolecularly with the b20-b21 CD4-binding loop in the outer domain, we hypothesized that the SLWDQ region of CD4 might replace its gp120 counterpart in the CD4-bound structure. Extensive molecular dynamic (MD) simulations corroborated this hypothesis. To experimentally verify the model, we introduced cysteine mutations in both Asp63 in the SLWDQ region of 4-domain soluble CD4 (sCD4) and Arg429 in the BG505-SOSIP.664 trimer, and the two mutated proteins were co-expressed in 293FS cells. Negative-staining electron microscopy (NSEM) and Western blot analyses under both reducing and non-reducing conditions documented the efficient formation of disulfide-bonded molecular complexes between the trimers and sCD4, thus validating the CD4 SLWDQ region as a contact surface for the gp120 b20-b21 loop. These results support the structural and functional mimicry between gp120 and CD4, which HIV-1 may exploit to optimize an intramolecular contact that is critical for stabilizing the inner-outer domain interaction in the pre-fusion envelope conformation.

P-C22

*The constitutive activation of NF- $\kappa$ B by HTLV-1 Tax-1 oncoprotein is inhibited by the MHC class II transactivator CIITA*

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Human T cell lymphotropic virus type 1 (HTLV-1) Tax-1, induces T cell transformation deregulating diverse cell signaling pathways. Tax-1 activates the NF- $\kappa$ B pathway through binding to NF- $\kappa$ B proteins and activation of the I $\kappa$ B kinase (IKK). Upon IKK-mediated phosphorylation of I $\kappa$ B and consequent I $\kappa$ B degradation, NF- $\kappa$ B migrates into the nucleus mediating Tax-1-stimulated gene expression. We show that the transcriptional regulator of major histocompatibility complex class II genes CIITA endogenously or ectopically expressed in different cells, inhibits the activation of the canonical NF- $\kappa$ B pathway by Tax-1 and we mapped the CIITA region that mediates this effect. CIITA affects the subcellular localization of Tax-1, which is mostly retained in the cytoplasm, and this correlates with the impaired migration of the NF- $\kappa$ B RelA subunit into the nucleus. Cytoplasmic and nuclear mutant forms of CIITA reveal that CIITA exploits different strategies to suppress Tax-1-mediated NF- $\kappa$ B activation in both sub-cellular compartments. CIITA interacts with Tax-1 without preventing Tax-1 binding to both IKK $\gamma$  and RelA. Nevertheless, CIITA affects Tax-1-induced IKK activity, causing the retention of the inactive p50/RelA/I $\kappa$ B complex in the cytoplasm. Nuclear CIITA associates with Tax-1/RelA in nuclear bodies, blocking Tax-1-dependent activation of NF- $\kappa$ B-responsive genes. Thus, CIITA inhibits both cytoplasmic and nuclear steps of Tax-1-mediated NF- $\kappa$ B activation. These results, indicate that CIITA is a versatile molecule that might also counteract Tax-1 transforming activity. Unveiling the molecular basis of CIITA-mediated inhibition of Tax-1 functions may be important in defining new strategies to control HTLV-1 spreading and oncogenic potential.



P-C23

*Spleen tyrosine kinase (Syk) controls IFN- $\alpha$  production inhibited in plasmacytoid dendritic cells by surface glycoproteins of HIV, HBV and HCV*

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Crosslinking of regulatory immunoreceptors, such as BDCA-2 (CD303) or ILT7 (CD85g), of plasmacytoid dendritic cells (pDCs) efficiently suppresses production of type-I interferon (IFN)- $\alpha/\beta$  and other cytokines in response to Toll-like receptor (TLR) 7/9 ligands. Viral surface glycoproteins, HIV-1 gp120, hepatitis C virus (HCV) E2 and hepatitis B virus (HBV) HBsAg were shown to be ligands of pDC regulatory immunoreceptors. Cytokine-inhibitory pathway triggered by ligation of regulatory immunoreceptors is mediated by spleen tyrosine kinase (Syk) associated with the ITAM-containing adapter of regulatory immunoreceptors. Here we demonstrate by pharmacological targeting of Syk that in addition to the negative regulation of TLR7/9 signaling via regulatory immunoreceptors, Syk also positively regulates the TLR7/9 pathway in human pDCs. Novel highly specific Syk inhibitor AB8779

suppressed IFN- $\alpha$ , TNF- $\alpha$  and IL-6 production induced by TLR7/9 agonists in primary pDCs and in the pDC cell line GEN2.2. Triggering of TLR9 or regulatory immunoreceptors signaling induced a differential kinetics of phosphorylation at Y352 and Y525/526 of Syk and a differential sensitivity to AB8779. Consistent with the different roles of Syk in TLR7/9 and regulatory immunoreceptors signaling, a concentration of AB8779 insufficient to block TLR7/9 signaling still released the block of IFN- $\alpha$  production triggered via the regulatory immunoreceptor pathway, including that induced by HIV, HBV and HCV. Thus, pharmacological targeting of Syk partially restored the main pDC function—IFN- $\alpha$  production. Opposing roles of Syk in TLR7/9 and regulatory immunoreceptor pathways may regulate the innate immune response to weaken inflammation reaction.





P-D1

*Transient stimulation of cells harboring latent proviruses contributes to the methylation of the HIV-1 promoter*

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Human immunodeficiency virus type 1 (HIV-1) latency represents the major barrier to virus eradication in infected individuals because cells harboring latent HIV-1 provirus are not affected by current antiretroviral therapy (ART). We previously demonstrated that DNA methylation of HIV-1 long terminal repeat (5' LTR) restricts HIV-1 reactivation and, together with chromatin conformation, represents an important mechanism of HIV-1 latency maintenance. Here, we explored the new issue of temporal development of DNA methylation in latent HIV-1 5' LTR. In the Jurkat CD4(+) T cell model of latency, we showed that the stimulation of host cells contributed to de novo DNA methylation of the latent HIV-1 5' LTR sequences. Consecutive stimulations of model CD4(+) T cell line with TNF- $\alpha$  and PMA or with SAHA contributed to the progressive accumulation of 5' LTR DNA methylation. Further, we showed that once established, the high DNA methylation level of the latent 5' LTR in the cell line model was a stable epigenetic mark. Finally, we explored the development of 5' LTR DNA methylation in the latent reservoir of HIV-1-infected individuals who were treated with ART. We detected low levels of 5' LTR DNA methylation in the resting CD4(+) T cells of the group of patients who were treated for up to 3 years. However, after long-term ART, we observed an accumulation of 5' LTR DNA methylation in the latent reservoir. Importantly, within the latent reservoir of some long-term-treated individuals, we uncovered populations of proviral molecules with a high density of 5' LTR CpG methylation. Our data showed the presence of 5' LTR DNA methylation in the long-term reservoir of HIV-1-infected individuals and implied that the transient stimulation of cells harboring latent proviruses may contribute, at least in part, to the methylation of the HIV-1 promoter.

P-D2

*Macrophage Autophagy Facilitates Sustained Release of Nanoformulated Antiretroviral Drugs From Autophagosomal Depots Enhances Anti-HIV-1 Activities*

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The realization that long-acting nanoformulated antiretroviral therapy (ART) can be broadly made available for clinical care is stymied by the few drugs available. Thus, there is an immediate need to develop sustained release anti-HIV products with a dosing interval measured in months. This would overcome inherent therapeutic challenges of drug adherence, biodistribution and long-term toxicities. We previously demonstrated that this goal could be realized by producing hydrophobic nanoformulated ART (nanoART) with particle sequestration in macrophage endosomes. This may be made possible through the actions of the mixed lineage kinase inhibitor, URM-099. The drug facilitates reductions in viral load and protects CD4+ T lymphocytes in infected humanized mice. We now report that URM-099's action is, in part, through the stimulation of autophagy. URM-099 reduces JNK phosphorylation leading to modulation of mTORC1, which in turn, controls vesicular trafficking through transcription factor EB (TFEB). TFEB affects antiretroviral drug (ARV) nanoparticle depots and drug-particle dissociations. Human monocyte-derived macrophages given both nanoformulated atazanavir (nanoATV) and URM-099 then infected with HIV-1ADA show TFEB nuclear translocation and mTORC1 inhibition. Parallel upregulation of LC3B, BECN1 and SQSTM1 are observed. URM-099 increases nanoATV levels in autophagosomes for a range of antiretroviral concentrations with improvements in mitochondrial activity and pharmacokinetic profiles. This leads to improved viral clearance and are operative for both ATV and dolutegravir. As treatment regimen adherence is a significant factor in ARV success URM-099's abilities to stimulate autophagy and extend ART actions may facilitate its use as a booster for regimens of long-acting ARVs.



P-D3

*Crystal structures of small-molecules HIV-1 entry inhibitor, BMS-378806 and BMS-626529, bound to BG505 SOSIP.664 HIV-1 Env trimer reveal an allosteric competitive entry inhibition mechanism.*

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The HIV-1-envelope (Env) spike is a conformational machine that switches between multiple prefusion conformations to a postfusion conformation to facilitate HIV-1 entry. Extensive interest has focused on the prefusion mature closed conformation, as it is the target of neutralizing antibodies. One hall-mark of the prefusion conformation is the recognition by the small molecule entry inhibitor, BMS-378806, and its derivative BMS-626529, currently in clinical trial. BMS-626529 has been shown to inhibit HIV infection with an EC50 of 1-10 nM in vivo but the mechanism of inhibition remains unclear. Here, we obtained the crystal structures of the soluble mimic of HIV-1 Env, BG505 SOSIP.664 bound by antibodies 35O22 and PGT122 and small molecule BMS-378806 and BMS-626529 at 3.8 and 3.7 Å resolution, respectively. The structures revealed the location and orientation of binding of these lead candidate HIV-1 entry inhibitors. They bind gp120 at a surface pocket adjacent to the Phe 43 cavity where CD4 inserts. The structures explained the resistance phenotypes reported. Together with biophysical and antigenic characterization, the structures also provide a mechanism of inhibition for these small molecules: they inhibit CD4 binding and CD4-induced conformational changes through an allosteric competitive mechanism. Moreover, the chemical details of BMS-378806 and BMS-626529 interactions should allow for their structure-based optimization.

P-D4

*Evaluation of Combination Long-Acting Nanoformulated Antiretroviral Therapy in HIV-1 Infected huPBL NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ MICE*

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Early clinical intervention with combination antiretroviral therapy (ART) in human immunodeficiency virus type one (HIV-1) infected people was demonstrated to have sustained benefits by lowering viral set points, protecting CD4+ T lymphocytes and limiting viral reservoir pools. All positive affect long-term patient morbidity and mortality. Notably and as shown in our prior studies cell-targeted nanoformulated ART (nanoART) improves pharmacokinetic and pharmacodynamics profiles and reduces local and systemic drug toxicities in experimental models of HIV/AIDS. These drugs are being considered in a step-wise drug regimen for viral eradication. In the present study, we administrated long-acting nanoformulated combinations of folic acid-decorated cabotegravir, lamivudine and abacavir to human peripheral blood lymphocyte (PBL) reconstituted NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice 24 or 72 hours after HIV-1 infection. Replicate mice received free drugs at equivalent doses. Animals were sacrificed two weeks after HIV-1ADA infection. NanoART-treated animals demonstrated 75% recovery of CD4+ T lymphocytes calculated from a total CD3+cell pool. Immunohistochemical tests showed HIV-1p24+cells (~80/1000HLA-DR+cells) only in untreated infected animals. NanoART suppressed HIV-1gag RNA expression to <10 copies by real-time RT-PCR in spleen, live, lung, brain and kidney in 90% animals while total viral DNA remained detectable. Limited proviral DNA was seen in nanoART treated animals with plasma viral RNA undetected (<20copies/ml). Early treatment of combination nanoART in infected PBL mice can effectively suppress HIV-1 growth while preventing CD4+ T lymphocyte loss.



P-D5

*Synthesis and characterization of core-shell silica cobalt ferrite nanoparticles as a first step towards developing ultrasensitive MRI probes for long-acting antiretroviral drug biodistribution testing*

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Cobalt ferrite (CF) nanoparticles were developed as magnetic resonance (MR) imaging contrast agents. However, its size, shape and chemical features also make the particles applicable for screening biodistribution of drug carriers. In developing long-acting nanoformulated antiretroviral drugs (ARVs), CF provides unique translational prospects. Thus, we investigated whether europium (Eu) doped CF nanoparticles (CFEu) could be useful in screening of nanoparticle ARVs. CFEUs were synthesized by a modified solvothermal technique and functionalized with core-shell-Pluronic®F-127 (Si-CFEu). The synthesized superparamagnetic, monodispersed CFEUs were highly crystalline with an inverse-spinel structure. The particle size was 7.2 nm and 140 nm for CFEu and Si-CFEu, respectively. Folic acid (FA) decoration onto the surface of the Si-CFEu facilitated mononuclear phagocyte (MP) targeting. Uptake and retention of FA-Si-CFEu nanoparticles by MP were demonstrated. The presence of intracellular particles was made by prussian blue and confocal microscopy confirming the ultrahigh transverse relaxivity ( $r_2=7917.9 \text{ s}^{-1} \text{ mL } \mu\text{g}^{-1}$ ) measurements. To simulate immune activation of HIV-1-infected person, rats were treated with 2 mg/kg lipopolysaccharide (LPS). MRI scans demonstrated increase in T2 and decrease in signal in the reticuloendothelial system of the LPS treated rats 24 h post-injection of Si-CFEu and FA-Si-CFEu. FA-Si-CFEu particles provided significantly higher tissue iron concentration compared to Si-CFEu signifying targeting abilities. These data bring us one step closer towards using decorated Si-CFEu particles to assess biodistribution of nanoformulated ARVs.

P-D6

*Lasting Reduction Of HIV Replication In Chronically Infected Humanized Mice (HSC-NSG) By Targeting Transcription With A CDK9 Inhibitor*

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Current "Shock and Kill" does not cure HIV and has the risks of seeding new waves of HIV infection. An alternative approach to "Shock and Kill" is to prevent infected cells from making new virus particles by targeting HIV transcription. However, inhibitors of HIV transcription are not currently available. We have previously demonstrated that pharmacological inhibition of cellular CDK9, a cofactor of the HIV Tat protein, with Indirubin 3'-monoxime (IM) inhibits HIV transcription in vitro. We have now evaluated the antiviral activity of IM in humanized mice (HSC-NSG model), chronically infected with HIV (BaL strain). Treatment with IM (5 mg/kg) or vehicle (control) was initiated at 5 weeks after infection and continued for 15 weeks. We measured HIV RNA and CD4/CD8 ratios in blood samples at different time points. On week 15 of treatment, mice treated with IM had a mean plasma HIV RNA of  $1.2 \times 10^3$  copies/ml, which was significantly lower than in control mice ( $2.1 \times 10^5$  copies/ml;  $P = 0.01$ ). Consistent with these decreases in HIV viremia, CD4/CD8 ratios were significantly higher in IM treated mice (mean CD4/CD8 of 4.05) than in controls (mean CD4/CD8 of 0.43;  $P = 0.03$ ). There was no significant difference in the weight of animals in IM treatment versus controls, suggesting treatment was not toxic. We also evaluated the toxicity of IM in immunocompetent Balb/c mice, and demonstrated that IM doses of up to 25 mg/kg (highest dose tested) did not cause hematologic, renal or liver toxicity. These data demonstrate the in vivo anti-HIV activity of IM, an inhibitor of HIV transcription. Studies are ongoing to determine if targeting HIV transcription could also impact the size of the HIV reservoir.





P-D7

*Synthesis and Characterization of a Long Acting Nanoformulated Dolutegravir*

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Dolutegravir (DTG) is a potent integrase strand transfer inhibitor with a unique resistance profile for treatment and prevention of the human immunodeficiency virus type one (HIV-1) infection. With immediate need for long acting antiretroviral drugs (ARVs) in treatment regimens, long acting prodrugs that reduce systemic metabolism and polarity, affect lipophilicity and enhance membrane permeability are of high value. To this end, we synthesized a modified DTG (MDTG) through esterification of the DTG 7-hydroxyl group with myristic acid then confirmed by <sup>1</sup>H-NMR and FT-IR spectroscopy. Poloxamer (P407) and folic acid (FA) decorated-P407 nanoformulations were synthesized for both DTG and MDTG to further improve its targeting potential and longevity. Stability, size, shape and charge of the developed nanoformulations were compared against their pharmacokinetic (PK) and biodistribution. All had uniform particle size (237-393 nm) and negative zeta potentials. The nanoformulated MDTG showed > 100-fold drug uptake in human monocyte-derived macrophages (MDM) and prolonged cell retention beyond 15 days when compared to native DTG. The results paralleled antiretroviral activities as evidenced by protection of MDM against HIV-1 challenge with the macrophage tropic ADA strain at days 1, 5, 10 and 15 after drug formulation treatment as measured by HIV-1 reverse transcriptase activity and p24 staining. PK testing showed that the drug's half-life was increased from 86 hours for P407-DTG to > 223 hours for P407-MDTG with projected drug levels above the IC<sub>90</sub> (64 ng/mL) for > 8 weeks. These results demonstrate early promise for development of a potent, long-acting parenteral formulation of DTG for the treatment of HIV-1 infection.

P-D8

*Macrophage exosomes as novel antiretroviral drug delivery platforms*

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Exosomes are 30-150 nm extracellular vesicles that transport RNAs, proteins and small molecules between cells. Exosomes serve as endogenous carriers of biological cargo with natural features for immune cell targeting, and thus have generated immense interest for drug delivery. Herein we tested two methods of drug loading into exosomes. First, we dosed human monocyte-derived macrophages (MDM) with native and nanoformulated atazanavir (nanoATV) and assessed drug loading into released exosomes. MDM showed avid abilities to produce drug-loaded nanoparticles over a period of 14 days. MDM dosed with native ATV secreted ~1.5 µg of ATV /109 exosomes whereas 500 ng of ATV/109 exosomes were secreted with nanoATV at day 1 and 3. At day 7 nanoATV treated MDM secreted ~1 µg of ATV in /109 exosomes while for native ATV loaded MDM drug levels were at or below limits of detection. The drug concentration was increased 1.5 fold when MDM were treated with URM-099, a mixed lineage kinase inhibitor known to potentiate macrophage nanoparticle sequestration. Second, drug loading into naïve exosomes ex vitro was investigated. Three methods of ATV incorporation were employed: sonication, freeze-thaw and incubation with 0.2% saponin. The freeze-thaw method resulted in the lowest drug loading, 11.3 µg / ml of exosomes. Incubation with saponin at RT increased loading to 19.2 µg / ml. Sonication allowed for the greatest drug loading, 57.9 µg / ml. Together we posit that exosomes can be harnessed as carriers for antiretroviral drugs with natural targeting machineries for macrophage delivery. As exosomes are known to cross blood brain barrier and known to reach lymphoid organs, this delivery system could be used to target HIV reservoirs.





P-D9

*Development of a Next Generation Long-Acting Nanoformulated Cabotegravir*

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Significant interest in long-acting parenteral (LAP) antiretroviral drugs (ARVs) has set a new bar for HIV/AIDS care. LAP ARVs are designed to improve treatment compliance and affect resistance patterns and drug toxicities. Cabotegravir (CAB), a potent viral integrase inhibitor, now in phase II clinical trials as an LAP (CAB-LAP) currently demonstrates sustained plasma drug levels in humans up to 52 weeks after single intramuscular dose. We sought that CAB LAP could be even further developed to reduce injection volumes and improve pharmacokinetic (PK) profiles that would make it even easier to administer. To this end a prodrug nanoformulation of CAB (called NMCAB) was made to improve drug half-life along with its antiretroviral activities. CAB was chemically conjugated to myristoyl chloride, transforming it into a more hydrophobic moiety. The prepared NMCAB particles were size reduced by high-pressure homogenization with poloxamer407. Uptake and retention were tested in human monocyte-derived macrophages (MDM). Antiretroviral activity was evaluated by reverse transcriptase (RT) activity and cell HIV-1p24 expression. NMCAB was efficiently taken up by cells with sustained release up to 10 days. Notably, the parent drug formulations were eliminated after a single day of treatment. MDM treated with NMCAB showed RT activity at or below the level of detection when infected at days 0, 2, 5 and 10 after drug particle treatment. HIV-1p24 was not detected in the NMCAB treated group at any of these time points. A PK evaluation is being monitored for two months after a single injection. We conclude that NMCAB shows improved uptake, retention and antiretroviral activity. Biodistribution, PK and pharmacodynamic studies are underway.

P-D10

*IL-18 Reconstitutes Vg9Vd2 T Cells in HIV+ Patient Samples*

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The Vg9Vd2 subset of human T cells is depleted during HIV disease and not reconstituted after prolonged antiretroviral therapy (ART). Their loss is part of the immunodeficiency syndrome likely linked to increased opportunistic infections or cancer, and normal Vg9Vd2 function was observed in HIV natural virus suppressors with little to no blood viremia in the absence of ART. Our goals are to understand the mechanisms preventing full reconstitution of Vg9Vd2 T cells and discover conditions in HIV+ patients that are limiting the functionality of existing cells. We know that prolonged ART increases the T cell receptor complexity of Vg9 chains in blood, showing that de novo cell synthesis is occurring in treated HIV patients. Unfortunately, these cells remain incapable of responding to prototypical phosphoantigens even while re-gaining responsiveness to aminobisphosphonate drugs including zoledronic acid (Zol). Zol treatment increases stimulatory IPP and promotes secretion of Caspase-1 processed cytokines IL-18 and IL-1 $\beta$  through its effects on the NLRP3 inflammasome. The Vd2 T cell subset was particularly high for IL-18 receptor expression compared to traditional IL-18 targets CD8+ T and natural killer cells. IL-18 stimulation increased proliferation, enhanced the accumulation of effector memory cells, and increased expression of TNFa and IFNg. Vd2 T cells from HIV+ PBMCs treated with IPP proliferated much more in the presence of IL-18. Expression of CD56 and NKG2D markers also increased with IL-18 stimulation. Our results focus on the direct effects of IL-18 on Vg9Vd2 T cells. We are currently testing for links between IL-18 deficiency and the failed IPP response of Vg9Vd2 T cells from HIV+ patients treated with ART. Ultimately, we hope to translate this study to promote recovery of V $\delta$ 2 T cells in immuno-compromised HIV patients for anti-tumor and anti-viral responses.



P-D11

*Live attenuated rubella/gag vectors elicit potent antibody and T cell responses that may change the course of SIV infection in macaques*

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Live attenuated viruses are among our most potent and durable vaccines. We have used the attenuated rubella vaccine strain RA27/3 as a live vector to express vaccine inserts such as HIV env and SIV gag. The vaccine strain has demonstrated its safety and potency in millions of young children; one or two doses protect for life against rubella. The host range of rubella includes rhesus macaques, and rubella/gag vectors are potently immunogenic. The antibody response to a single dose of rubella/gag was as great as natural SIV infection. The antibodies persisted for over one year and declined at the same rate as anti-rubella antibodies. Titers were boosted strongly by a second dose of vector. Following a DNA prime and rubella/gag boost, macaques elicited high levels of T cells specific for MHC class I restricted Gag epitopes. The response to CM9 in Mamu A01 animals reached 4 to 8% of circulating CD8+ T cells. The T cell response persisted over 8 months and was boosted on re-exposure to the vectors. Rubella vectors have a number of desirable properties for use in a "monkey cure" experiment. Rhesus macaques can be infected with SIV, followed within days by ART treatment. Then, while on ART, the macaques can be immunized with a DNA vaccine prime and rubella/gag boost. Once the T cells have peaked, ART will be interrupted. We expect viral rebound in the control group within 3 to 4 weeks. The vaccine group may control virus replication and prevent viral rebound. Or they may have viral "blips" that become less frequent over time. If the animals establish immune control, we will study them for complete viral eradication or "functional cure" with residual virus. The results will indicate whether early ART plus a vaccine can produce immunological control of infection. The outcome will be important for the nearly 20,000 babies who are born with HIV infection each month. They may indicate the level of T cell response that predicts control. The same vector grows well in macaques and man. It is likely that a positive result in macaques could be translated into human vaccine design.

P-D12

*DNA-PK Inhibition Potently Suppress HIV Transcription, Replication and Proviral Reactivation*

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DNA-dependent protein kinase (DNA-PK), a nuclear protein kinase that requires association with DNA for its kinase activity, plays important roles in the regulation of different DNA transactions, mainly transcription, replication and DNA repair. We reported DNA-PK facilitated HIV transcription by interacting with and phosphorylating the carboxyl terminal domain (CTD) of the RNA polymerase II (RNAP II) complex recruited to HIV LTR. In our current study, we found that besides catalyzing directly CTD phosphorylation, DNA-PK mediates the recruitment of P-TEFb at HIV LTR. Accordingly, DNA-PK inhibition via highly specific small molecule inhibitors resulted in severe impairment of the phosphorylation of the serine 2 and serine 5 of the RNAP II CTD. Chromatin immunoprecipitation (ChIP) analysis showed that DNA-PK inhibition led to the establishment of transcriptionally repressive heterochromatin structures at the HIV LTR. Consequently, we found strong restriction to HIV transcription and replication by DNA-PK inhibitors. Similarly results were also obtained upon DNA-PK knockdown, validating the direct effect of inhibitors on DNA-PK. Moreover, we found that DNA-PK inhibitors successfully limit the reactivation of latent HIV proviruses in patients' PBMCs. This observation presents a strong evidence for the inclusion of transcription inhibitors, such as DNA-PK inhibitors as supplements to HAART regimens, in order to further enhance the restriction of HIV replication, besides limiting transcription from proviruses and resultant deleterious effects from viral proteins, such as in CNS. Thus, our results can be exploited for HIV "Cure" Research with Emphasis on Viral Suppression.



P-E1

*Immunologic Criteria are Poor Predictors of Virologic Outcomes: Implications for HIV Monitoring in a Large Treatment Program in Nigeria*

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Background: Nigeria has made significant gains in scaling-up access to HIV prevention, treatment and support services and by the end of 2014, provided antiretroviral therapy (ART) to over 747,382 individuals and care and support to almost 1.5 million people. The main objective of this study was to determine predictors of immunologic failure in the absence of routine viral load monitoring. Methods: This was a retrospective cohort study of 12,456 HIV-infected patients enrolled into HIV care at the University of Abuja Teaching Hospital (UATH) between February, 2005 and December, 2014. To identify predictors of immunologic failure, univariate and multivariate analyses were performed using log binomial models to estimate relative risks (RR) and confidence intervals. All available plausible predictors were included in the multivariate models if they were significant at a P value of <.20. Results: Among 2,602 patients with immunologic failure, 868 (33.3%) had VL measurements and 381 (43.9%) of these had a detectable VL. Fifty six samples (56/198; 28%) had no resistance; 160 (80%) harbored NRTI resistance; 151 (76.3%) M184I/V; 29 (14.6%) had  $\geq 3$  TAMs, and 37 (18.7%) had K65R, of which all were on TDF. One hundred and sixty-two samples (81.8%) harbored NNRTI resistance; 72 (36.4%) Y181C and 68 (34.3%) K103N with 53 % having  $\geq 2$  etravirine associated mutations. Service entry point [RR (95%CI): 0.79 (0.64–0.91);  $p < 0.001$ ]; being on NVP containing regimen [RR (95%CI): 1.21 (0.99 – 1.45);  $p = 0.023$ ]; WHO stage III or IV [0.76 (0.60 – 0.96);  $p = 0.013$ ]; baseline CD4 cell count  $< 200$  cells/ul [0.19 (0.16 – 0.22);  $p < 0.001$ ]; and male gender [1 (1.07 – 1.40);  $p = 0.005$ ] were associated with immunologic failure. Conclusions: Immunological criteria for failure erroneously classified patients without virological replication as failing therapy in our program. Patients with both virologic and immunologic failures had extensive accumulations of drug resistance mutations.

P-E2

*Co-occurrence Network Analysis of Adverse Events for Typhoid Fever Vaccines in VAERS*

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Salmonella enterica serotype Typhi is considered as one of the high-priority potential bioterrorism agents by the Center for Disease Control and Prevention (CDC). Vaccines against Typhi can help with the prophylaxis against typhoid fever. However, little effort has been conducted for post-market safety monitoring of typhoid fever vaccines. In this paper, we proposed a novel network-based computational approach to investigate the co-occurrence relationships among adverse events reported after typhoid fever vaccine (TYP). We focused on association data that were recorded in the Vaccine Adverse Event Reporting System (VAERS) between 1990 and 2014. First, we extracted and summarized adverse event (AE) information from TYP related reports in the VAERS database using Resource Description Framework (RDF). Then, we applied a series of network approaches to the AE co-occurrence network to identify potential associations among these AEs. Specifically, we (1) constructed an AE co-occurrence network after the typhoid fever vaccines; (2) calculated network properties of AE co-occurrence network; (3) identified condensed subnetworks in AE co-occurrence network; and (4) compared MedDRA terms associated with AEs in each subnetwork. We observed that (1) AE co-occurrence network shares the same scale-free network property as other biological networks and social networks; (2) AEs clustered in one subnetwork are usually enriched in certain MedDRA terms.



P-E3

*HCV Treatment Lost to Follow-up: Comparing HIV/HCV infected and HCV infected Patients*

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Background: Direct-acting antivirals have high rates of SVR, however, there remain significant challenges to completing treatment. HIV/HCV infected patients are already linked to care and presumably are less likely to be lost to follow-up during HCV treatment. Methods: Data from two groups in the observational study ASCEND were analyzed; those lost to follow-up post consent but prior to starting LDV/SOF (n=81) and those lost during treatment (n=57), in order to determine the effect of HIV/HCV infection on lost to follow up rates. RESULTS: 81 patients were lost to follow-up post consent; 15 HIV/HCV infected and 66 HCV infected. Over 50% of both cohorts were unreachable despite multiple attempts (53% (n=8) HIV/HCV infected and 55% (n=36) HCV infected). Of the 600 who were started on LDV/SOF between May and November 2015, 143 were HIV/HCV infected (1 tri-infected) and 457 were HCV infected (2 HCV/HBV). 57 patients were lost to follow-up while on treatment; 15 (10%) HIV/HCV infected and 42 (9.2%) HCV infected (P=0.62). There was also no difference in adherence to visits for week 4, 8 and 12 during treatment between the two cohorts. Conclusions: Our results show that HIV/HCV infected patients are just as likely as HCV infected patients to be lost to follow-up during treatment. Engaging all patients in care through SVR12 requires vigorous outreach regardless of current linkage to care. Strategies focused on retention in care should remain a priority.

P-E4

*The Community HIV Epidemic Control (CHEC) model: A novel integration of community and facility care to achieve 90-90-90 in Zambia*

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Introduction: As drugs, funding, and ethics align for universal treatment of HIV-infected persons worldwide, demand for service threatens to exceed existing healthcare infrastructure. Developing countries need novel methods to diagnose HIV-infected people, recruit them into care, and achieve long-lasting viral suppression. Methods: The University of Maryland Community HIV Epidemic Control (CHEC) model is an innovative community-based approach to the HIV care continuum. A community health worker, equipped with a tablet linked to the national electronic health record, offers HIV testing and health messaging to all members of the community. Persons testing HIV+ are referred to a healthcare facility and followed up to ensure they are in care and receiving treatment; any HIV-exposed infants are followed through the testing cascade. CHEC currently provides Option B+ to pregnant women, community ART based on national guidelines, and community ART with a Test and Start approach. Results: To date CHEC has been implemented in over 100 sites in Zambia, with more than 400 CHWs trained and deployed. In the first year of CHEC deployment, HIV testing rose from 21,051 to 71,289 clients (339% increase), of whom 20,623 (29%) were tested in the community. Major PMTCT indicators, such as ANC testing, HIV+ pregnant women on ART, and HIV-exposed infant testing, all increased three- to five-fold. Initial cost analysis shows that after initial startup, one CHW can follow 500 clients for \$4-5 per client per year. Conclusion: The CHEC model represents a novel and cost-efficient community-based approach to achieving the UNAIDS 90-90-90 goals of epidemic control and offers a durable solution to healthcare system strengthening in sub-Saharan Africa.





P-E5

*Correlates of Facility Delivery among HIV+ Women in Rural North-Central Nigeria: Findings from the INSPIRE MoMent Study*

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**INTRODUCTION:** Facility-based delivery and timely access to PMTCT services are key for HIV-free survival for HIV-exposed infants. In Nigeria, little is known about this indicator and its correlates among HIV+ women, especially in rural areas. We evaluated factors associated with facility-based delivery among HIV+ pregnant women enrolled in a large prospective cohort study. **METHODS:** Demographic & obstetric data were analyzed. Correlates of facility-based delivery were evaluated using chi square test, and odds ratios were determined. **RESULTS:** Delivery data was available for 430 of the 496 women enrolled in 9 districts across 2 states in rural North-Central Nigeria. Overall, 62.8% of women delivered at facility, and 57.6% of women were newly HIV-diagnosed. Median age for women delivering at and outside facilities was identical (27 yrs). Only 19.2% of study participants were primigravidae. Compared to multigravid women, primigravidae were more likely to deliver at facility (OR 2.1, CI 1.2 to 3.7). None of the other evaluated factors (education, religion, gestational age at booking, new or previous HIV diagnosis) correlated with facility delivery. **DISCUSSION:** Our data suggest that only primigravidity was associated with facility delivery among rural HIV+ women. However, other factors not evaluated eg socioeconomic, commuting distance and availability of 24/7 delivery services at the local health facility may be correlates as well. The high rate of facility deliveries in our study, among HIV+ women, (>60%) compared to that of the general Nigerian population (<40%) suggests that the diagnosis of HIV and initiation of ART, in and of themselves, facilitate hospital births among pregnant women, even in rural areas where healthcare utilization is poor. **CONCLUSIONS:** Determinants of facility delivery among HIV+ women may be multifactorial and interdependent. Early ART initiation, especially among primigravid HIV+ women, may encourage facility delivery in Nigeria's rural PMTCT program.

P-E6

*Investigating user preference for mobile app versus paper for data collection in a large survey of health facilities in north central Nigeria*

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**Introduction:** Direct digital input avoids multistep manual entry from hard-copy documents into electronic platforms. This aims to limit data entry errors & improve efficiency. E-tool use in public health is increasing but often faces user resistance. We evaluated preferences for manual vs e-data collection among data collectors (DCs) at the Institute of Human Virology Nigeria. **Method:** This was a crossover design study. Data collected was from assessments of comprehensive HIV service in primary healthcare facilities (HCF). DCs were trained on both manual and electronic (mobile app) methods. Upon assessing half of their assigned HCFs with one modality, DCs switched to the 2nd entry method. After HCF assessment, a 22-item questionnaire was used to survey DC preferences. A user satisfaction score was calculated & variances in preference analyzed using Chi-square & multinomial logistic regression. **Results:** A total of 168 HCFs in 3 states were assessed by 76 DCs. Randomization allotted 48.9% to app & 51.6% to paper. A total of 55 (72.3%) DCs responded. Mean age of respondents was 41.8y; 51.9% had Masters/PhD, 42.6% had Bachelors' degrees and 3.7% had lower certification. Approximately 95% owned a smartphone but only 18.2% routinely managed digital databases. Overall, 50.0% of DCs preferred the app; 20.4% paper, 18.1% preferred both & 11.1% had no preference. Perception of 'higher quality' was 49.1% for app and 27.3% for paper. Ratings for user friendliness and speed of entry were similar for both methods. Preference for app was associated with post-secondary education (p=0.009) but not age, gender or database experience. With a Net Promoter Score of 36, 50.9% of users were active promoters of e-entry, 34.5% were passive promoters, & detractors, 14.5%. **Conclusion:** Mobile apps for HIV program data entry is feasible, acceptable & considered higher quality versus paper. User acceptability & dexterity should be assessed prior to its implementation for data collection.



P-E7

*Maternal HAART Predicts Favorable Early Infant Diagnosis outcome in North-Central Nigeria*

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**Introduction:** Early Infant Diagnosis (EID) that yields a negative HIV result is the desired pediatric outcome of Prevention of Mother to Child Transmission (PMTCT) interventions. Pregnancy and breastfeeding are considered critical infectivity periods for most of Nigeria's 380,000 HIV positive children. We explored predictors of EID outcomes among offspring of PMTCT enrollees across 5 north central states, who had virologic polymerase chain reaction testing between October 2015 and May 2016. **Method:** Important clinical data accompany EID samples. Samples are clustered from multiple health facilities and processed at PEPFAR supported regional public health labs. Composite data from a single regional lab was obtained and binomial logistic regression performed to ascertain the effects of several factors on the likelihood of infants achieving a negative HIV DNA-1 result. Independent variables explored were: Female and male sex, breastfeeding status, maternal treatment regimen: prophylaxis, Highly Active Anti-Retroviral Treatment (HAART) during, or before index pregnancy. **Results:** A total of 3373 (93.7%) samples were analyzed. 45.7% were female. Mean age of Infants' at sample collection was 2.12 weeks. The full model with all the predictors was statistically significant  $\chi^2(8) = 175.18$ ,  $p < .001$ , indicating ability to differentiate those with successful and failed EID outcomes. The model explained between 4.8% (Cox & Snell R Square) and 15% (Nagelkerke R<sup>2</sup>) of the variance in EID outcomes and correctly classified 95.2% of the cases. However, only 2 variables were statistically significant; Maternal HAART during pregnancy and HAART before pregnancy. Controlling for other factors in the model, HAART before pregnancy and during pregnancy were 11 (OR=10.93) & 7 (OR=6.53) times more likely respectively, to have HIV negative infants compared to prophylaxis regimen. **Conclusion:** HAART prior to and during pregnancy is associated with an increased likelihood of achieving a favorable EID result.

P-E8

*Differential expression of innate immune response genes among chronic hepatitis B virus clinical phases*

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In the present study, we investigated the innate immune gene expression among clinical phases of CHB including immune tolerant (IT); immune active (IA); inactive carrier (IC); and hepatitis B e antigen (HBeAg)-negative (ENEG) hepatitis disease phases and healthy control as well. To this end, expression of IFN type I, II, III, their receptor subunits, IRFs, TLRs and other IFN induced genes in peripheral blood mononuclear cells (PBMCs) from categorized CHB patients into four groups were compared with each other as well as healthy donors. Forty HBsAg positive treatment naïve subjects were enrolled in this study. They were positive for HBsAg at least for six mounts and without coinfection with HIV, HCV and HDV. Total and Viral RNA isolated from PBMCs and serum, respectively. In addition to viral load and biochemical tests, expression of 37 genes including IFN genes, IFN receptor genes, TLRs, IRFs and a number of other Interferon-Inducible genes were subjected to qPCR. The highest response of innate immune system has been observed in the IT and ENEG phases and the IC phase had lowest response; 31 genes of 37 studied genes had maximum rate of mRNA expression in IT and ENEG and minimum expression level of 23 genes has been found only in the IC phase. The maximum mRNA expression level among IFNs, IFN receptor subunits, IRFs and TLRs genes in all clinical phases belongs to IFN $\lambda$ 2 and 3, IFN $\gamma$ R2, IRF7 and TLR7 and their minimum level of mRNA expression had been observed in IFN $\alpha$ , IFN $\lambda$ R1, IRF8 and TLR2 respectively. Finally, we can conclude that innate immune response genes are expressed differentially between chronic HBV phases and this difference may help to develop new precious and non-invasive methods to determine the progression of disease among patients suffering from chronic HBV.



P-E9

*Racial Disparity in All-Cause Mortality Among HCV-Infected Individuals in a General US Population, NHANES III*

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**Background.** There are few long-term nationally representative studies of all-cause mortality among those infected with hepatitis C virus (HCV). When an additional five years of data were made publicly available in 2015, the Third National Health and Nutrition Examination Survey (NHANES III) Linked Mortality File became the longest nationally representative study in the United States. Our objective was to update the estimated HCV-associated all-cause mortality in the general US population and determine any differences by sex, age, and race/ethnicity. **Methods.** HCV status was assessed in 9,117 nationally representative adults aged 18-59 years from 1988 to 1994, and mortality follow-up of the same individuals was completed through 2011 and made publicly available in 2015, in The NHANES III Linked Mortality File. **Results.** There were 930 deaths over a median follow-up of 19.8 years. After adjusting for all covariate risk factors, chronic HCV infection had 2.63 times (95% CI: 1.59-4.37; P=0.0002) higher all-cause mortality rate ratio (MRR) compared with being HCV-negative. All-cause MRR was stratified by sex, age, and race/ethnicity. Only race/ethnicity was a significant effect modifier of MRR (P<0.0001) as the highest MRR of chronic HCV compared to HCV-negative was 7.48 (95% CI 2.15-26.10, P=0.001) among Mexican-Americans, 2.67 (95% CI 2.67-5.56, P=0.009) among non-Hispanic whites, and 2.02 (95% CI 1.20-3.40, P=0.007) among non-Hispanic blacks. **Conclusions.** Racial disparity was seen in the all-cause mortality as Mexican-Americans with chronic HCV had approximately seven times higher mortality rate than HCV-negative individuals. This suggests that these at-risk individuals should be targeted for HCV screening and treatment, particularly given the availability of the new highly effective HCV therapies.

P-E10

*The effect of HCV co-infection on the rate of development of complications among HIV infected elite controllers*

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**Background:** Elite controllers are HIV-infected patients capable of naturally suppressing HIV-1 to undetectable levels without therapy. Approximately 20% of people infected with HIV are co-infected with hepatitis C (HCV). The purpose of this study was to estimate the effect of HCV co-infection among HIV infected individuals on the time to the development of complications compared to HIV mono-infected individuals. **Methods:** A retrospective cohort study of the Institute of Human Virology elite controller cohort was conducted to estimate the relationship between HCV co-infection and the rate of complications. **Results:** Of the 65 elite controller patients enrolled in the NVS cohort since 2004, 55 were included in the study sample. 25 (45%) patients were HIV/HCV co-infected and 30 (55%) were HIV mono-infected. Patients co-infected with HCV experienced significantly more complications than HIV mono-infected patients (p=0.002). Patients co-infected with HCV had 4.35 (95% CI 1.56 - 12.19) times the hazard for the development of complications compared to HIV mono-infected patients (there was no significant difference when HCV related complications were excluded). After controlling immune activation (cellular and sCD14), patients co-infected with HCV experienced complications at 3.47 (1.20 - 10.05) times the rate of HIV mono-infected patients. **Discussion:** HCV co-infected patients experience complications at significantly higher rates compared to HIV mono-infected patients after controlling for immune activation. HCV and/or HAART therapy may prove beneficial in decreasing morbidity and mortality rates in these patients, particularly among those co-infected with HCV.





P-E11

*CD8+CD28-T cells: A major T cell subset with diverse functions in chronic HBV infection*

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CD8+T cells, considered as key players in viral clearance, are functionally attenuated in chronic HBV infection (CHI). A prominent feature of CD8+T cells in CHI is the downregulation of CD28 molecule that is essential for T cell activation. Different studies had assigned diverse functional attributes to these CD8+CD28-T cells in various disease contexts. The present study focused on phenotypic and functional characterization of CD8+CD28-T cells in CHI to gain an insight into their contribution in disease pathogenesis. We observed significant accumulation of CD8+CD28-T cells in both HBeAg positive (e+) and HBeAg negative (e-) CHB patients as compared to inactive carriers (IC) and healthy subjects. Flow cytometry analysis of CD8+CD28-T cells revealed significantly (about 1.5 fold) high expression of FoxP3, IL-10, TGF- $\beta$  and low CD127 expression than their CD8+CD28+T cell counterparts, in e(+) and e(-) CHB patients, suggesting their enhanced immunosuppressive activity. Concurrently, a profound (almost 4-6 times) increase in expression of perforin, granzyme, NKG2D, CD57, IFN- $\gamma$  and TNF- $\alpha$  in CD8+CD28-T cells from CHB was seen, as compared to CD8+CD28+T cells denoting their high cytolytic predisposition. Our data depicted a comprehensively greater fold change in expression of cytotoxic proteins than that of suppressive markers by CD28-T cells as compared to CD28+T cells, suggesting a predominant cytolytic nature of CD28-T cells. CXCR3 expression on both CD28 counterparts was comparable implying analogous predilection of both to be recruited to liver. Thus CD8+CD28-T lymphocytes represent a major and a primarily cytotoxic T cell subset which might potentially contribute to disease severity in active hepatitis.

P-E12

18th Annual International Meeting of the Institute of Human Virology

*Drug Resistance Mutations among HIV-Positive Nigerian Children on 2nd Line ART*

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**INTRODUCTION:** For Nigerian children, access to new generation ARVs, viral load (VL) & drug resistance testing (DRT) are limited. Without DRT, 2nd line failure is difficult to identify & treat. Knowledge of common drug resistance mutations (DRMs) may guide empiric salvage therapy. We evaluated for DRMs among a cohort of HIV+ children on 2nd line ART. **METHODS:** This study was conducted at a large urban ART clinic. Registers identified children on 2nd line ART who had failed NNRTI or ABC/3TC/AZT. Profile data were obtained from charts, & blood samples were processed for VL & DRT in a central lab. Failure was defined as VL  $\geq$  1000 copies/ml after  $\geq$ 6 mos on ART. VL was performed with Roche Cobas AmpliPrep/TaqMan at a limit of 20 copies/ml. DRMs were defined using the 2013 IAS list. **RESULTS:** There were 973 children on ART at the clinic; 65/973 (6.7%) on 2nd line; 15 had died, transferred, or lost to follow up. Blood samples were obtained from 47/50 children of which 62% were male. Median duration on 1st line and 2nd line ART were 4.6 and 1.8 y respectively & 95% of children had received ART for  $\geq$ 6 m. For 88% of children, first line ART was NVP-based. All children received LPV/r-based 2nd line ART, with 41.5%, 34.2%, 14.6% receiving ABC+3TC, TDF+XTC, & AZT+3TC NRTI backbones respectively. Twelve of 47 samples had VL  $>$ 1000 & received DRT. All 4 major PI DRMs detected (M46I, I50V, I54V, V82M) were found in 1 sample. Detections for NNRTI DRMs were in 4/12 samples for K103N & A98G, 3/12 for V179E & Y181C, & in 2 for V90I & G190A. For NRTI DRMs, M184V was found in 7/12 samples; M41L & T215 in 3, & K219Q, D67H, K70R, T69N in 2 samples. **DISCUSSION:** Given prior regimens, high frequency of M184V/M41L/T215 and K103N/A98G/Y181C were expected. No K65 DRMs were found, suggesting potential use of TDF or ABC in salvage, barring other key TAMs. Despite universal LPV/r use, only 1 sample had major PI DRMs. LPV/r & ABC or TDF use after  $>$ 6 months shows low selection of key DRMs. **CONCLUSIONS:** Including LPV/r+ABC or TDF in salvage may be helpful; however, new, suppressive, child-friendly drugs are needed.

P-E13





P-E14

*An observational cohort of post-natal HIV transmission among women on HAART who breastfeed their infants in Nigeria: Findings from the INFANT Study*

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**Introduction:** Infants born to HIV-infected mothers, who do not acquire HIV in utero or perinatally, continue to be at risk of acquiring HIV through breastfeeding. The World Health Organization (WHO) guidelines recommend exclusive breastfeeding and HAART. Mothers with normal pregnancy and babies were enrolled. We analyse a cohort of mother-infant pairs in Nigeria and calculate HIV incident rate among breast-fed infants. The INFANT study is an observational cohort. Participants were recruited from Plateau State Specialist Hospital in Jos, Nigeria and followed for 2 years (April 2013 to March 2015). Mothers were included in the study their infants were  $\geq 36$  weeks of gestation and had a birth weight  $\geq 2.4$ kg. All infants infected in utero or at peripartur were excluded from the study. A survival analysis was used to calculate the incident rate of mother to child transmission. **Discussion:** A total of 384 HIV+ mother and infants were included in this analysis. The mothers' median age was 30 years. About 120 (31%), 153 (40%), 101 (26%) of the cohort had primary, secondary and higher education respectively. Almost all were married 370 (96%), 177 (46%) were self employed, and 352 (92%) reported living in a house but only 161 (42%) has refrigeration. Almost all mothers 383 (98%) exclusively breast at birth but the numbers dropped with each passing month. A reasonable number of mothers were not virologically suppressed, but only 2 infants were infected over the 2-year study period, giving this site an overall incidence of HIV-infection was 0.5/100 person-years (95% CI:0.06-1.81). **Conclusion:** The study of post-natal mother to child transmission is highly relevant especially with the availability of HAART. This study has shown a low incidence rate of transmission via breastfeeding proving that breastfeeding is a safe and recommended practice for mothers living with HIV in our African setting.

P-E15

*Performance Evaluation of HIV Test Kits from 20 Countries to Determine their Suitability as Claimed by Manufacturers*

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**Background:** HIV test kits are used throughout the world as a primary measure to protect the blood supply and provide diagnosis to save lives. The US government purchases large numbers of HIV tests from a variety of manufacturers at considerable expense. **Objective:** To evaluate a large number and variety of HIV test kits from many countries to determine if they meet the claims of the manufacturers. **Methods:** From September 2010 through June 2016, a total of 666 lots of HIV test kits from 14 manufacturers were received at the IHV from over 20 countries. The performance of each test kit was assessed for sensitivity, specificity, and precision using panels of sera that included positives for HIV-1 and HIV-2, negatives, and HIV-1 seroconversion panels. Test kits were also assessed for their ability to perform accurately at the higher range of allowed temperature (28C) to challenge the kits at temperatures that are

not uncommon in many countries. **Results:** Of the 666 HIV test kit lots, 662 (99.4%) successfully passed the evaluation with perfect performance. Of the 4 lots from two manufacturers that did not pass, 3 were found to produce high background that interfered and 1 gave substantial false-positive results. In one case, the failure resulted in cessation of bulk purchase of test kits by the US Government and removal from WHO's e-catalogue. In one case, a country reported poor performance while the test passed the evaluation, resulting in a visit to the country to assess activities that were subsequently found to be unsuitable. **Conclusion:** In our evaluation of a large number and variety of HIV test kit lots from 14 manufacturers and from over 20 countries, nearly all performed perfectly and met the manufacturers' claims.