Table of Contents

Daily Schedule .................................................. 3
Organization ..................................................... 4
Contributors ..................................................... 5
Networking ....................................................... 6
Schedule at a Glance .......................................... 7
Program Schedule ............................................. 10
Abstracts ......................................................... 42
Author Index ................................................... 128
Daily Schedule

Monday, May 12, 2014
- Women in Science
- D&D 101: Synergies Between Chemistry and Biology in Antiviral Drug Design – Lessons From the Trenches
- Keynote Address
- Opening Reception

Tuesday, May 13, 2014
- HBV Symposium
- Tuesday Lunch (on your own)
- Elsevier Authors’ Workshop (lunch provided)
- Gertrude Elion Award Lecture
- New Drug Screening Technologies and Emerging Infections
- In Vitro Evaluation and Drug Resistance
- Poster Session I
- New Member and First Time Attendee Networking

Wednesday, May 14, 2014
- Antonin Holý Award Lecture
- Medicinal Chemistry
- Antiviral Targets and Mechanism of Action
- Wednesday Lunch (on your own)
- ISAR Business Meeting
- Research Triangle Park Session
- Poster Session II
- ICAR Career Networking and Discussion

Thursday, May 15, 2014
- William Prusoff Young Investigator Award Lecture
- Challenges in HIV Infection, Treatment and Prevention
- Thursday Lunch (on your own)
- Animal Models of Infection
- Clinical Evaluation of Antiviral Therapies
- Shotgun Presentations
- Closing Banquet

Friday, May 16, 2014
- EUVIRNA Session
International Society for Antiviral Research AND
Twenty-Seventh International Conference on Antiviral Research

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Rhonda Cardin

The International Society For Antiviral Research (ISAR)
The Society was organized in 1987 as a non profit scientific organization for the purpose of advancing and disseminating knowledge in all areas of antiviral research. To achieve this objective, the Society organizes an annual meeting. The Society is now in its twenty fifth year of existence, and has approximately 550 members representing 30 countries. For membership application forms or further information, please contact Dr. Graciela Andrei, Secretary, ISAR at the address noted above. Membership application forms will also be available at the Conference Registration desk, or from our website www.isar-icar.com.
Contributors TO THE

27th International Conference on Antiviral Research
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Tokyo, Japan
Vertex Pharmaceuticals, Inc.
Cambridge, MA, USA
Keynote Address

ERADICATION THERAPIES FOR HIV: BUILDING THE CRITICAL PATH
David Margolis, M.D.
University of North Carolina

HIV PREVENTION 2014-2021: MANAGING ASPIRATION AND EXPECTATION
Myron Cohen, M.D.
University of North Carolina

Monday, May 12, 2014
4:45 PM – 6:30 PM
LOCATION: BALLROOM A

Networking Events

WOMEN IN SCIENCE LUNCH*
Monday, May 12, 2014
11:30 AM – 2:00 PM
LOCATION: 305 A
*Space is limited and pre-registration is required.

OPENING RECEPTION
Monday, May 12, 2014
6:30 PM – 8:30 PM
LOCATION: BALLROOM LOBBY

ELSEVIER AUTHORS’ WORKSHOP
(Lunch provided)
Tuesday, May 13, 2014
12:00 PM – 1:00 PM
LOCATION: ROOM 305A

NEW MEMBER AND FIRST TIME ATTENDEE NETWORKING EVENT
Tuesday, May 13, 2014
6:15 PM – 7:30 PM
LOCATION: ROOM 305A

CAREER NETWORKING AND DISCUSSION
Wednesday, May 14, 2014
6:00 PM – 7:45 PM
LOCATION: ROOM 305A

CONFERENCE BANQUET
Thursday, May 15, 2014
Reception 7:00 PM
Dinner 7:30 PM – 10:00 PM
LOCATION: BALLROOM LOBBY/ BALLROOM A
## Monday, May 12, 2014

<table>
<thead>
<tr>
<th>TIME</th>
<th>EVENT</th>
<th>LOCATION</th>
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<tbody>
<tr>
<td>11:00 am – 5:30 pm</td>
<td>Registration</td>
<td>BALLROOM LOBBY</td>
</tr>
<tr>
<td>11:30 am – 2:00 pm</td>
<td>Women In Science Roundtable</td>
<td>305 A</td>
</tr>
<tr>
<td>2:00 pm – 4:15 pm</td>
<td>Drug Discovery and Development 101</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>4:45 pm – 5:00 pm</td>
<td>Greetings and Opening Remarks</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>5:00 pm – 6:30 pm</td>
<td>Keynote Address</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>6:30 pm – 8:30 pm</td>
<td>Opening Reception</td>
<td>BALLROOM LOBBY</td>
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<td>Light hors d’oeuvres served</td>
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## Tuesday, May 13, 2014

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<tr>
<th>TIME</th>
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<tbody>
<tr>
<td>7:00 am – 5:00 pm</td>
<td>Registration</td>
<td>BALLROOM LOBBY</td>
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<tr>
<td>8:00 am – 12:00 pm</td>
<td>HBV Symposium</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>12:00 pm – 1:00 pm</td>
<td>Elsevier Authors’ Workshop (Lunch Provided)</td>
<td>305 A</td>
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<tr>
<td>12:00 pm – 1:30 pm</td>
<td>Lunch</td>
<td>On Your Own</td>
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<tr>
<td>1:30 pm – 2:30 pm</td>
<td>Gertrude Elion Award Lecture</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>2:30 pm – 3:15 pm</td>
<td>New Drug Screening Technologies and Emerging Infections</td>
<td>BALLROOM A</td>
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<tr>
<td>3:45 pm – 4:30 pm</td>
<td>In Vitro Evaluation and Drug Resistance</td>
<td>BALLROOM A</td>
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<tr>
<td>4:30 pm – 6:00 pm</td>
<td>Poster Session I</td>
<td>BALLROOM C</td>
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<tr>
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<td>Light hors d’oeuvres served</td>
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<tr>
<td>6:15 pm – 7:30 pm</td>
<td>New Member and First Time Attendee Networking</td>
<td>305 A</td>
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<td>Light hors d’oeuvres served</td>
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## Schedule at a Glance

### Wednesday, May 14, 2014

<table>
<thead>
<tr>
<th>TIME</th>
<th>EVENT</th>
<th>LOCATION</th>
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<tbody>
<tr>
<td>7:30 am – 5:00 pm</td>
<td>Registration</td>
<td>BALLROOM LOBBY</td>
</tr>
<tr>
<td>8:30 am – 9:15 am</td>
<td>Antonin Holý Award Lecture</td>
<td>BALLROOM A</td>
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<tr>
<td>9:15 am – 10:15 am</td>
<td>Medicinal Chemistry</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>10:45 am – 12:00 pm</td>
<td>Antiviral Targets and Mechanism of Action</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>12:00 pm – 1:30 pm</td>
<td>Lunch</td>
<td>On Your Own</td>
</tr>
<tr>
<td>1:30 pm – 2:00 pm</td>
<td>ISAR Business Meeting</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>2:00 pm – 4:30 pm</td>
<td>Research Triangle Park</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>4:30 pm – 6:00 pm</td>
<td>Poster Session II</td>
<td>BALLROOM C</td>
</tr>
<tr>
<td>6:00 pm – 7:45 pm</td>
<td>ICAR Career Networking and Discussion</td>
<td>305 A</td>
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### Thursday, May 15, 2014

<table>
<thead>
<tr>
<th>TIME</th>
<th>EVENT</th>
<th>LOCATION</th>
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<tbody>
<tr>
<td>7:30 am – 5:00 pm</td>
<td>Registration</td>
<td>BALLROOM LOBBY</td>
</tr>
<tr>
<td>8:30 am – 9:15 am</td>
<td>William Prusoff Young Investigator Award</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>9:15 am – 12:00 pm</td>
<td>Challenges in HIV Infection, Treatment and Prevention</td>
<td>BALLROOM A</td>
</tr>
<tr>
<td>12:00 pm – 1:30 pm</td>
<td>Lunch</td>
<td>On Your Own</td>
</tr>
<tr>
<td>1:30 pm – 2:30 pm</td>
<td>Animal Models of Infection</td>
<td>BALLROOM A</td>
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<tr>
<td>3:00 pm – 4:00 pm</td>
<td>Clinical Evaluation of Antiviral Therapies</td>
<td>BALLROOM A</td>
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<tr>
<td>4:00 pm – 4:30 pm</td>
<td>Shot Gun Presentations</td>
<td>BALLROOM A</td>
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<tr>
<td>7:00 pm – 7:30 pm</td>
<td>Closing Reception</td>
<td>BALLROOM LOBBY</td>
</tr>
<tr>
<td>7:30 pm – 10:00 pm</td>
<td>Conference Banquet and Program</td>
<td>BALLROOM A</td>
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### Friday, May 16, 2014

<table>
<thead>
<tr>
<th>TIME</th>
<th>EVENT</th>
<th>LOCATION</th>
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<tbody>
<tr>
<td>7:30 am – 12:00 pm</td>
<td>Registration</td>
<td>ROOM 304 LOBBY</td>
</tr>
<tr>
<td>8:30 am – 12:00 pm</td>
<td>EUVIRNA</td>
<td>ROOM 304</td>
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</table>
Please join us for an informal career discussion and networking at the 27th ICAR meeting. This year we will again host an excellent group of moderators who are recognized experts in various areas of antiviral research and have pursued successful career in academia, government, or industry. As a part of the informal group discussion, the moderators will be ready to share their experience, answer questions, and provide feedback about career development. In addition, you will have opportunity to network with your colleagues and make new contacts.

The event is open to all ICAR attendees. Please sign up during the ICAR conference at the registration desk before 4 pm on Wednesday, May 14.

Refreshments will be served

Moderators

ACADEMIA SECTOR MODERATORS
- Jennifer Moffat, Ph.D. (SUNY Upstate Medical University, USA)
- Johan Neyts, Ph.D. (Rega Institute, Belgium)

GOVERNMENT SECTOR MODERATORS
- Gerardo Garcia-Lerma, Ph.D. (CDC, USA)
- Christopher Tseng, Ph.D. (NIAID/NIH, USA)

BIOTECH SECTOR MODERATORS
- M. Javad Aman, Ph.D. (Integrated Biotherapeutics, USA)
- Klaus Klumpp, Ph.D. (Novira, USA)

MID-SIZE PHARMA SECTOR MODERATORS
- Joy Feng, Ph.D. (Gilead, USA)
- Raj Kalkeri, Ph.D. (Vertex, USA)

LARGE PHARMA SECTOR MODERATORS
- Steve Ludmerer, Ph.D. (Merck, USA)
- Jun Tang, Ph.D. (GlaxoSmithKline, USA)

CONTACT RESEARCH ORGANIZATIONS (CRO) SECTOR MODERATORS
- James Noah, Ph.D. (Southern Research Institute, USA)
- Eric Stavale, Ph.D. (Integrated Biotherapeutics, USA)
**Monday, May 12, 2014**

**WOMEN IN SCIENCE ROUNDTABLE**  
*Chair(s): Amy Patick, Ph.D.*  
**ROOM 305A**  
**11:30 am – 2:00 pm**

**D&D 101: SYNERGIES BETWEEN CHEMISTRY AND BIOLOGY IN ANTIVIRAL DRUG DESIGN – LESSONS FROM THE TRENCHES**  
*Chair(s): Robert Buckheit, Ph.D.*  
**BALLROOM A**  
**2:00 pm – 4:15 pm**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Speaker</th>
<th>Affiliation</th>
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<tbody>
<tr>
<td>2:00 pm</td>
<td>1. Applications of Bioisosteres in Antiviral Drug Design.</td>
<td>Nicholas Meanwell, Ph.D.</td>
<td>Bristol-Myers Squibb</td>
</tr>
<tr>
<td>2:30 pm</td>
<td>2. Antiviral Proteins from Natural Product Extracts: from Discovery to Development.</td>
<td>Barry O’Keefe, Ph.D.</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>3:00 pm</td>
<td>3. The Role of Molecular Modeling in Drug Discovery.</td>
<td>Andrea Brancale, Ph.D.</td>
<td>Cardiff University</td>
</tr>
<tr>
<td>3:30 pm</td>
<td>4. Fragment Based Screening and Its Role in Drug Discovery.</td>
<td>Nickolay Chirgadze, Ph.D.</td>
<td>X-CHIP Technologies, Inc</td>
</tr>
<tr>
<td>4:00 pm</td>
<td>Panel Discussion.</td>
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**COFFEE BREAK**  
**BALLROOM LOBBY**  
**4:15 pm – 4:45 pm**
KEYNOTE ADDRESS
Chair(s): Robert Buckheit, Ph.D.
BALLROOM A
4:45 pm – 6:30 pm

5:00 pm  5. Eradication Therapies for HIV: Building the Critical Path.
         David Margolis, M.D.
         University of North Carolina

         Myron Cohen, M.D.
         University of North Carolina

OPENING RECEPTION
BALLROOM LOBBY
6:30 pm – 8:30 pm

Tuesday, May 13, 2014

HBV SYMPOSIUM
Chair(s): William Delaney, Ph.D., Andy Cuconati, Ph.D.
BALLROOM A
8:00 am – 12:00 pm

8:00 am  7. Hepatitis B Treatment: Challenges and Opportunities.
         Anna Lok, M.D.
         University of Michigan, USA

8:30 am  8. The Hepatitis B Virus Life Circle: Recent Achievements and Challenges.
         Stephan Urban, M.D.
         University of Heidelberg Germany

9:00 am  9. Immune Regulation and Co-Stimulation in HBV-Infected Patients: an Uneasy Truce.
         Kyong-Mi Chang, Ph.D.
         University of Pennsylvania, USA

9:30 am  10. Diversifying the Hepatitis B Pipeline: Current Efforts to Explore Novel Mechanisms.
          Andy Cuconati, Ph.D.
          Institute for Hepatitis & Virus Research, Pennsylvania Commonwealth Institute, USA
COFFEE BREAK  
BALLROOM LOBBY  
10:00 am – 10:30 am

10:30 am  11. Targeting cccDNA to Cure Chronic Hepatitis B.  
Massimo Levrero, Ph.D.  
Sapienza Universita’ di Roma, Italy

10:55 am  12. HBV Capsid Protein: Biology and Potential as a Drug Target for Antivirals.  
Adam Zlotnick, Ph.D.  
University of Indiana, USA

John Morrey, Ph.D.  
Utah State University, USA

Stephan Menne, Ph.D.  
Georgetown University, USA

TUESDAY LUNCH (on your own)  
12:00 pm – 1:30 pm

ELSEVIER AUTHORS’ WORKSHOP (lunch provided)  
ROOM 305A  
12:00 pm – 1:00 pm

GERTRUDE ELION AWARD LECTURE  
Chair(s): Phil Furman, Ph.D.  
BALLROOM A  
1:30 pm – 2:30 pm

1:30 pm  15. Collaborative Antiviral Studies for the Discovery of Drugs to Treat Cytomegalovirus Infections.  
John Drach, Ph.D.  
University of Michigan, USA

NEW DRUG SCREENING TECHNOLOGIES AND EMERGING INFECTIONS  
Chair(s): Jinhong Chang, M.D., Ph.D., Mike Bray, M.D.  
BALLROOM A  
2:30 pm – 3:15 pm

2:35 pm  16. Organotypic Epithelial Raft Cultures as Versatile and Faithful Environments for Antiviral Drug Testing.  
Thomas R. Broker, Jei-Hwa Yu, Hsu-Kun Wang, Nilam Sanjib Banerjee, Louise T. Chow  
University of Alabama at Birmingham, Birmingham, Alabama, United States
2:45 pm 17. Identification of Human Neutralizing Antibodies Against MERS-CoV and Their Role in Virus Adaptive Evolution.
Sudhakar Agnihotram¹, Xian-Chun Tang², Yongjun Jiao², Jeremy Stanhope², Rachel Graham¹, Eric Peterson², Ralph Baric¹, Wayne Marasco²
¹Departments of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States, ²Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, Massachusetts, United States

Brian B. Gowen¹, Jonna B. Westover¹, Eric J. Sefing¹, Kevin W. Bailey¹, David Safronetz², Yousuke Furuta³, Donald F. Smee¹
¹Utah State University, Logan, Utah, United States, ²Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, United States, ³Toyama Chemical Co., Ltd., Tokyo, Japan

3:05 pm 19. Evaluation of Luciferase and GFP-Expressing Nipah Viruses for Rapid Quantitative Antiviral Screening.
Michael K. Lo, Stuart T. Nichol, Christina F. Spiropoulou
Centers for Disease Control and Prevention, Atlanta, GA, United States

COFFEE BREAK
BALLROOM LOBBY
3:15 pm – 3:45 pm

IN VITRO EVALUATION AND DRUG RESISTANCE
Chair(s): Tomas Cihlar, Ph.D. and Graciela Andrei, Ph.D.
BALLROOM A
3:45 pm – 4:30 pm

3:45 pm 20. Antiviral Optimization of a HCV NS5A Inhibitor Zn6168 with Picomolar Pan-Genotypic Activity and Excellent Safety.
Zheng-Yun James Zhan¹,², Qing Li², Guoyan Zhang², Hua Yan¹
¹AB Pharma Ltd., Shanghai, China, ²Zannan SciTech Co., Ltd., Shanghai, China

3:54 pm 21. Activation of Intracellular Viral Sensors by the Anti-Hepatitis Agent SB 9200 – Implications for Broad-Spectrum Antiviral Activity.
R.P. Iyer¹, A. Sheri¹, R.K. Pandey¹, S. Padmanabhan¹, B.E. Korba², S. Bose³, M.E. Cunningham⁴, G.R. Foster⁴
¹Spring Bank Pharmaceuticals, Milford, MA, United States, ²Georgetown University Medical Center, Division of Molecular Virology and Immunology, Washington, DC, United States, ³University of Texas Health Sciences Center, San Antonio, TX, United States, ⁴The Liver Unit, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, England, United Kingdom

4:03 pm 22. RSV Envelope Pseudotyping and Cell-Cell Fusion Assays Enable Rapid Phenotypic Analysis of RSV Fusion Protein Resistance Mutations.
Kirsten Stray, Shreya Pramanick, Krista McCutcheon, Robert Jordan, Tomas Cihlar, Michel Perron
Gilead Sciences, Inc., Foster City, CA, United States
Program Schedule

4:12 pm  23. Expressed Drug-Resistant HIV Subpopulations Identified by Surface Marker Immunocapture.

S Malik¹, L Morris², C Yang³, C Zeh¹, J Kiarie⁴, J Stringer⁵,⁶, P Weidle¹, J Johnson¹
¹Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, United States, ²National Institute for Communicable Diseases, Johannesburg, South Africa, ³Division of Global HIV/AIDS, Centers for Disease Control and Prevention, Atlanta, GA, United States, ⁴Kenyatta National Hospital, Nairobi, Kenya, ⁵CIDR, Lusaka, Zambia, ⁶U North Carolina, Chapel Hill, NC, United States

4:21 pm  24. The Mode of Action of ST-246 Primarily Involves F13L in Orthopoxviruses and Also B5R in Camelpox Virus.

Sophie Duraffour¹, Maria M. Lorenzo², Gudrun Zöller³, Dimitri Topalis¹, Dennis E. Hruby⁴, Rafael Blasco², Hermann Meyer³, Graciela Andrei¹
¹Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium, ²Departamento de Biotecnologia, INIA, Madrid, Spain, ³Bundeswehr Institute of Microbiology, Munich, Germany, ⁴SIGA Inc, Corvallis, Oregon, United States

POSTER SESSION I (ABSTRACTS 25-73, 75-80)
BALLROOM C
4:30 pm – 6:00 pm

25. Emergence of Genetically Variant Hepatitis C Virus Population in Patients with Low Viral Loads (< 250 IU/mL), Pakistan.

Muhammad Ali¹, Muhammad Idrees²
¹University of The Punjab, Lahore, Punjab, Pakistan

26. Discovery and In Vivo Evaluation of PI4KIIib Inhibitors for Picornavirus Infections.

Martin Andrews¹, Dale Mitchell², Herve van der Poel¹, Hilde van der Schaar¹, Frank Van Kuppeveld³, Pieter Leyssen², Johan Neyts², Armando da Palma³
¹Galapagos NV, Mechelen, Belgium, ²BioFocus plc, Chesterford Research Park, United Kingdom, ³Rega Institute, Leuven, Belgium, ⁴Nijmegen University, Nijmegen, Netherlands

27. Glycine Ester Derivatives of 2H-Pyran-3-Carboxylic Acid as Possible Anti-HSV Agents.

Chandralata Bal¹, Srinivas Karampuri¹, Durbadal Ojha², Paromita Bag², Debprasad Chattopadhyay², Ashoke Sharon¹
¹Department of Applied Chemistry, Ranchi, Jharkhand, India, ²ICMR Virus Unit, ID & BG Hospital, Kolkata, West Bengal, India

28. Furthering Our Understanding HIV-1 Tropism in Late Stage Disease.

Maria M Bednar¹,², L Ping¹,², SB Joseph¹,², LP Kincer¹,², MS Cohen¹,³, R Swanstrom¹,²,⁴
¹UNC Center of AIDS Research, Chapel Hill, NC, United States, ²Lineberger Comprehensive Cancer Center, Chapel Hill, NC, United States, ³Division of Infectious Diseases, School of Medicine, Chapel Hill, NC, United States, ⁴Department of Biochemistry and Biophysics, Chapel Hill, NC, United States
29. Decreased Ring Size in Cyclotriazadisulfonamide (CADA) Analogs with Preserved CD4 Down-Modulating and Anti-HIV Activity.
   Thomas W. Bell, Emily D. Scarbrough, Victor Van Puyenbroeck, Dominique Schols, Kurt Vermeire
   1Department of Chemistry, University of Nevada, Reno, NV, United States, 2K U Leuven Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium

   Kimberley S. Benschop, Marieke Hoogerwerf, Harrie van der Avoort, Erwin Duizer, Marion P. Koopmans
   1National Institute for Public Health and the Environment, Bilthoven, UT, Netherlands, 2Erasmus Medical Center, Rotterdam, ZH, Netherlands

31. Cytotoxicity and Antiviral Activity of a New Fluorine-Containing Derivatives of 1,2,3-Triazoles.
   Liubov Biliavska, Svitlana Zagorodnya, Olga Povnitsa, Yurii Shermolovich, Ganna Gudz, Nadiya Nesterova
   1Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukrenia, 2Institute of Organic Chemistry NAS of Ukraine, Kyiv, Ukrenia

32. Development of a Duogel for Vaginal and Rectal Delivery of Microbicide Products.
   AD Boczar, CA Buchholz, L Yang, S Nugent, A Ham, CS Dezzutti, KW Buckheit, RW Buckheit Jr.
   1ImQuest BioSciences Inc, Frederick, MD, United States, 2Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, United States

   B Bogomolny, V Barzinsky, T Grydina, A Fedchuk, L Mudryk, V Lozitsky
   1Corporation Informational Medicine, Kyiv, Ukrenia, 2Mechnikov Ukrainian Anti-Plague Research Institute, Odesa, Ukrenia

34. Use of the ICCA to Predict Dosing of HIV Microbicide Products Required for Virus Sterilization in Target Tissue.
   CA Buchholz, KW Buckheit, C Shetler, CS Dezzutti, PM Mesquita, BC Herold, RW Buckheit Jr.
   1ImQuest BioSciences, Frederick, MD, United States, 2Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, United States, 3Albert Einstein College of Medicine, Bronx, NY, United States

35. Development of Tandemers of the Antiviral Natural Product Griffithsin as HIV Prevention Agents.
   KW Buckheit, RW Buckheit Jr., L Yang, AD Boczar, CA Buchholz, T Moulaei, A Alexandre, BR O’Keefe
   1ImQuest BioSciences Inc., Frederick, MD, United States, 2Protein Structure Section, Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, MD, United States, 3Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, MD, United States
   Ana M. Chamoun-Emanuelli, Zhilei Chen
   Texas A&M University, College Station, TX, United States

37. Synthesis of Unsymmetrical Cyclotriazadisulfonamide (CADA) Analogs as Human CD4 Down-Modulating Antivirals.
   Reena Chawla1, Victor Van Puyenbroeck2, Dominique Schols2, Kurt Vermeire2, Thomas W. Bell1
   1Department of Chemistry, University of Nevada, Reno, NV, United States, 2K U Leuven
   Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium

38. Identification of FDA-Approved Drugs That Inhibit Middle East Respiratory Syndrome Coronavirus Replication in Cell Culture.
   Adriaan de Wilde1, Dirk Jochmans2, Clara Posthuma1, Jessika Zevenhoven-Dobbe1, Martijn van Hemert1, Bernadette van den Hoogen3, Johan Neyts2, Eric Snijder1
   1Department of Medical Microbiology, Leiden University Medical Center, Leiden, ZH, Netherlands, 2Rega Institute for Medical Research, KU Leuven, Leuven, VBR, Belgium, 3Viroscience, Erasmus Medical Center, Rotterdam, ZH, Netherlands

   Thiago Dinis de Oliveira1, Anna Rath1,2, Andrea Rentmeister2, Chris Meier1
   1University of Hamburg, Hamburg, Germany, 2University of Münster, Münster, Nordrhein-Westfalen, Germany

40. Prodrugs for the Delivery of Anti-Filoviral N-Alkyldeoxynojirimycin Derivatives
   Yanming Du1, Richard Lu1, Fang Guo2, Ju-Tao Guo2, Timothy Block1,2, Bill Kinney1, Xiaodong Xu1, Jinhong Chang2
   1Baruch S. Blumberg Institute, Doylestown, Pennsylvania, United States, 2Drexel University College of Medicine, Doylestown, Pennsylvania, United States

41. Discovery of Inhibitors of Middle East Respiratory Syndrome Coronavirus Infection.
   Julie Dyall1, Christoph C. Coleman2, Brit J. Hart1, Monique Laidlaw4, Lisa M. Johansen4, Peter B. Jahrling1,3, Lisa E. Hensley1, Matthew B. Frieman2
   1IRF NIAID/NIH, Frederick, MD, United States, 2University of Maryland School of Medicine, Baltimore, MD, United States, 3EVPS NIAID/NIH, Frederick, MD, United States, 4Zalicus Inc., Cambridge, MA, United States

42. New Indolylarylsulfones as Potent and Broad Spectrum HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors.
   Valeria Famiglini1, Giuseppe La Regina1, Antonio Coluccia1, Andrea Brancale2, José A. Esté3, Romano Silvestri1
   1Sapienza University, Roma, RM, Italy, 2Cardiff University, Cardiff, CF, United Kingdom, 3Universitat Autònoma de Barcelona, Badalona, B, Spain

43. Synthesis of 2’-O,4’-C-Alkylene-Bridged Ribonucleosides and Their Evaluation as Inhibitors of HCV NS5B Polymerase.
   Rebecca Glen1, Benjamin A Mayes2, Stephen Moore1, Adel Moussa2, Alistair Stewart2
   1Peakdale Molecular Ltd, Chapel-en-le-Frith, High Peak, United Kingdom, 2Idenix Pharmaceuticals, Cambridge, MA, United States
44. Development of a High-Throughput Cell-Based Antiviral Assay for Screening Inhibitors of Chikungunya Virus.

Edwin Gong¹, Jean-François Bonfanti², Guenter Kraus¹
¹Janssen Infectious Diseases-Diagnostics BVBA, Johnson & Johnson Corporation, Beerse, Belgium, ²Janssen Research & Development, Val de Reuil Cedex, France


Jason D Graci¹, Stephen P Jung¹, Guangming Chen¹, Chunling Wang², Mildred Galvez², Zhengxian Gu¹, Eva Harris², Joseph M Colacino¹
¹PTC Therapeutics, Inc., South Plainfield, NJ, United States, ²Division of Infectious Diseases, School of Public Health, University of California, Berkeley, Berkeley, CA, United States

46. Discovery of Novel HCV IRES Inhibitors by Shape-Directed Screening.

David Grawoig¹, Carly Sherrod², Fethullah Karabiber³, Oleg V. Favorov⁴, Dirk P. Dittmer², Kevin M. Weeks¹
¹Chemistry Department, UNC-Chapel Hill, Chapel Hill, North Carolina, United States, ²Microbiology and Immunology Department, UNC-Chapel Hill, Chapel Hill, North Carolina, United States, ³Department of Computer Engineering, Yildiz Technical University, Istanbul, Turkey, ⁴Biomedical Engineering, UNC-Chapel Hill, Chapel Hill, North Carolina, United States

47. A Cell-Based High Throughput Assay for the Discovery of Compounds with Antiviral or Innate Immune Response-Modulating Activity.

Fang Guo¹, Xuesen Zhao¹², Yanming Du², Andrea Cuconati², Michael Goetz³, Timothy M. Block¹²³, Ju-Tao Guo¹, Jinhong Chang¹
¹Drexel University College of Medicine, Doylestown, PA, United States, ²Baruch S. Blumberg Institute, Hepatitis B Foundation, Doylestown, PA, United States, ³Natural Products Discovery Institute, Hepatitis B Foundation, Doylestown, PA, United States


Anthony Ham¹, William Lustig¹, Sean Nugent¹, David Katz², Charlene Dezzutti³, Ashlee Boczar¹, Karen Buckheit¹, Robert Buckheit¹
¹ImQuest BiSciences, Frederick, MD, United States, ²Duke University, Durham, NC, United States, ³Magee Womens Research Institute, Pittsburgh, PA, United States

49. Development of Fullerene Poly-Aminocaproic Acid for the Inhibition of Human Immunodeficiency Virus Type 1.

Tracy Hartman¹, Lu Yang¹, Amanda Helfrick¹, Lev Rasnetsov², Robert Buckheit¹
¹ImQuest Biosciences, Frederick, Maryland, United States, ²CJSC Intelpharm, Nizhny Novgorod, Russia

50. The Discovery of Novel Bioactive Small Molecules Targeting the Priming Complex of HIV-1.

Tracy Hartman², Richard Guenther¹, Sam Yenne¹, Steve Peterson¹, Robert Buckheit, Jr.², Daniel D. Sternbach¹
¹TRANA Discovery Inc., Cary, North Carolina, United States, ²ImQuest Biosciences, Frederick, Maryland, United States
Matthew K. Howe1, Brandt Levitt2, Brittany L. Speer1, Timothy A. Haystead1
1Department of Pharmacology and Cancer Biology, Durham, NC, United States, 2Department of Molecular Genetics and Microbiology, Durham, NC, United States

52. Comparative Analysis of Cell-Based Assays to Screen Small Molecules for HIV Reactivation.
David Irlbeck1, Robert Ferris1, Eugene Stewart2, Katrina Creech3, Aaron Goetz3, Kevin Brown1, Cristin Galardi1, David Favre1
1HIV Discovery Performance Unit, GlaxoSmithKline, Research Triangle Park, NC, United States, 2Computational Chemistry, GlaxoSmithKline, Research Triangle Park, NC, United States, 3Biological Sciences, GlaxoSmithKline, Research Triangle Park, NC, United States

53. AVI-7288 Provides Significant Survival Benefit for NHP Marburg Virus Infection and Is Safe in Human Volunteers.
Patrick Iversen1,2, Alison Heald1, Travis Warren3, Jay Wells3, Pete Sazani1, Amy Shurtleff3, Lisa Welch3, Sina Bavari3
1Sarepta Therapeutics, Cambridge, MA, United States, 2Oregon State University, Corvallis, OR, United States, 3United States Army Medical Research Institute of Infectious Disease, Fort Detrick, MD, United States

54. Determination of Antiviral Effects on BKV Replication In Salivary Gland Cells.
Liesl K Jeffers-Francis, Raquel A Burger-Calderon, Jennifer Webster-Cyriaque
University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

55. Assessing the Potential for Mitochondrial Toxicity of Ribonucleoside Analogs.
Zhinan Jin, Jerome Deval, Julian A. Symons, Kenneth Shaw, Gary Wang, Natalia Dyatkina, Leonid Beigelman, David B. Smith
Alios BioPharma, South San Francisco, CA, United States

56. Genetic and Phenotypic Analysis of Seasonal Influenza Viruses in South Korea from 2011/2012 to 2013/2014 Seasons.
Chi-Kyeong Kim, Mi-Sun Kim, Su-Jin Kim, Hyuk Chu, Jang-Hoon Choi, Joo-Yeon Lee, Soonyoung Han, Chun Kang
Division of Influenza Viruses, Center for Infectious Diseases, National Institute of Health, Korea CDC, Cheongwon-gun, Chungbuk, South Korea

57. News in the Development of 5-Azacytosine-Based Antivirals.
Marcela Krecmerova1, Alice Chupikova1, Miroslav Otmar1, Graciela Andrei2, Jan Balzarini2, Robert Snoeck2
1Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic, 2Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium
58. Characterization of an HIV Entry Inhibitor Targeting a gp120-gp41 Interface.
Daniel Leaman¹, Edward Murray², PJ Klasse³, Michael Zwick¹
¹TSRI, La Jolla, CA, United States, ²Pfizer, Sandwich, United Kingdom, ³Cornell, NY, NY, United States

V Lozitsky¹, A Fedchuk¹, T Grydina¹, S Basok², L Mudryk¹, L Shitikova¹, L Socheslo¹
¹Ukrainian I.I. Mechnikov Anti-Plague Research Institute, Odesa, Ukrenia, ²A.V.Bogatsky
Physic-Chemical Institute, Odesa, Ukrenia

60. Synthesis and Antimicrobial Evaluation of Some Novel 1-Naphtyl Acetic Acid Derivatives
Hossein Mostafavi¹, Elham soleymani Sani Dogja¹, Peyman Zare²
¹Faculty Of Chemistry University of Tabriz, Tabriz, Azarbaijan, Iran, ²Faculty of Chemistry, Tabriz, Azarbaijan, Iran

61. Synthesis and Antimicrobial Activity of Novel Succinimides Derivatives
Hossein Mostafavi¹, Zahra Taghizadeh¹, Haedeh Mobeyen²
¹Faculty Of Chemistry University of Tabriz, Tabriz, Azarbaijan, Iran, ²Faculty of Chemistry, Tabriz, Azarbaijan, Iran

62. Modeling of Wide Spectrum Anti-Influenza Activity Using HiT QSAR.
Eugene Muratov¹², Anatoly Artemenko¹, Ekaterina Varlamova¹, Liudmila Ognichenko¹, Stepan Basok¹, Elena Alekseeva¹, Alla Fedtchuk³, Victor Kuz'min¹
¹A.V.Bogatsky Physical-Chemical Institute NAS of Ukraine, Odessa, Ukrenia, ²University of North Carolina, Chapel Hill, North Carolina, United States, ³I.I. Mechnikov Ukrainian Anti-Plague Research Institute, Odessa, Ukrenia

Tobias Nack¹, Jan Balzarini², Chris Meier¹
¹Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany, ²Rega Institute for Medical Research, Katholieke University of Leuven, Leuven, Belgium

64. A Single 96-Well Plate Format for Evaluation of HBV Replication in Multiple Cell Lines.
Todd B. Parsley, Lu Yang, Robert W. Buckheit Jr.
ImQuest BioSciences, Frederick, MD, United States

65. Using Yeast-Based Platform for Discovery of Drugs Against Dengue Host Factors.
Kunj Pathak, Daniel Engel
Microbiology, UVA, Charlottesville, VA, United States
66. New 1-Phenyl-5-(1H-Pyrrol-1-Yl)-1H-Pyrazole-3-Carboxamides Inhibit Hepatitis C Virus Replication and Suppress the Expression of Cyclooxygenase-2.
Sveva Pelliccia1, Dinesh Manvar2, Giuseppe La Regina1, Johan Neyts3, Neerja Kaushik-Basu2, Romano Silvestri1
1Sapienza University, Roma, RM, Italy, 2New Jersey Medical School, Newark, New Jersey, United States, 3Katholieke Universiteit Leuven, Leuven, B, Belgium

67. Brincidofovir (BCV, CMX001) and Acyclovir (ACV) are Additive Against Cytomegalovirus (CMV) In Vitro.
Dean W Selleseth1, Phiroze B Sethna1, Mark N Prichard2, Randall Lanier1
1Chimerix, Inc., Durham, NC, United States, 2University of Alabama, Birmingham, AL, United States

68. Determination of Hepatitis C Based Using the Anti-HCV and HCV Ag.
Nafija Serdarevic
University of Sarajevo, Sarajevo, Bosnia and Herzegovina

69. In Vitro Selection of Brincidofovir-Resistant and Cidofovir-Resistant Human Adenovirus.
Phiroze Sethna, Andrew Bae, Dean Selleseth, Randall Lanier
Chimerix Inc., Durham, NC, United States

70. α-Pyranone Carboxamide: a New Scaffold Optimization as Possible Anti-HCV Agent.
Ashoke Sharon1, Ananda K Konreddy1, Massaki Toyama2, Wataru Ito2, Masanori Baba2, Chandralata Bal1
1Department of Applied Chemistry, Birla Institute of Technology, Ranchi, Jharkhand, India, 2Center for Chronic Viral Diseases, Kagoshima University, Kagoshima, Kagoshima, Japan

71. In Vitro and In Vivo Study of Lamivudine Sustained Release Tablet Using Modified Pea Starch.
Akhilesh Vikram Singh
Department of Materials Engineering, Indian Institute of Science, Bangalore, Karnataka, India

Uma S. Singh1, Ram C. Mishra1, Ravi Shankar1, Masaya Sugiyama1, Rajgopal Govindarajan1, Brent Korba3, Yasuhiro Tanaka2, Chung K. Chu1
1The University of Georgia, College of Pharmacy, Athens, GA, United States, 2University Graduate School of Medical Sciences, Nagoya, Japan, 3Georgetown University Medical Center, Washington, DC, United States

73. Properties of Novel Substances Based on Plant Flavonoids and Mechanisms of Their Antiviral Activity.
D.B Starosyla1, I.V. Gomolyako2, M.O. Platonov3, O.V. Vasylychenko1, M.Yu. Obolenskaja3, Yu. Porva1, S.L. Rybalko1, V.P. Lozitsky4
1L.V.Gromashevsky Institute of Epidemiology and Infectious Diseases of NAMS of Ukraine, Kyiv, Kiev, 2The O.O.Shalimov Nationality Institute of Surgery and Transplantation NAMS of Ukraine, Kyiv, Kiev, 3Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Kiev, 4I.I.Mechnikov Ukrainian Anti-Plague Research Institute of the Ministry of Health of Ukraine, Odessa, Odessa
75. Hepatitis B Virus Replication Is Blocked by N-Hydroxyisoquinolinedione Inhibitors of the Viral Ribonuclease H Activity.

John E Tavis\textsuperscript{1}, Catherine Cai\textsuperscript{1}, Elena Lomonosova\textsuperscript{1}, Eileen Moran\textsuperscript{1}, Xiaohong Cheng\textsuperscript{1}, Fabrice Bailly\textsuperscript{2}, Philippe Cotelle\textsuperscript{2}, Marvin J Meyers\textsuperscript{1}

\textsuperscript{1}Saint Louis University, Saint Louis, MO, United States, \textsuperscript{2}University of Lille, Lille, France

76. Fitness and Virulence of a Coxsackievirus Mutant That Can Bypass the Need for Host Cell Factor PI4KIII\(\beta\).

Hendrik Jan Thibaut\textsuperscript{1,2}, Hilde M. van der Schaar\textsuperscript{1,3}, Kjerstin H.W. Lanke\textsuperscript{3}, Pieter Leyssen\textsuperscript{2}, Martin Andrews\textsuperscript{3}, Johan Neyts\textsuperscript{2}, Frank J.M. van Kuppeveld\textsuperscript{1,3}

\textsuperscript{1}University of Utrecht, Utrecht, Netherlands, \textsuperscript{2}University of Leuven, Leuven, Belgium, \textsuperscript{3}Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

77. Discovery of Novel Acylguanidine-Based Small Molecules That Block Influenza A M2 Ion Channel Activity and Drug-Resistant Virus.

Ian Tietjen\textsuperscript{1}, Scott C Miller\textsuperscript{1}, Daniel C Kwan\textsuperscript{1}, Brent Johnson\textsuperscript{2}, Hannah E Boycott\textsuperscript{1}, Doug Chou\textsuperscript{1}, David D Busath\textsuperscript{2}, David Fedida\textsuperscript{1}

\textsuperscript{1}University of British Columbia, Vancouver, BC, Canada, \textsuperscript{2}Brigham Young University, Provo, UT, United States

78. An Enterovirus 71 Mouse Model with Central Nervous System Involvement.

Aloys Tijsma, Hendrik Thibaut, David Franco, Johan Neyts

Rega Institute for Medical Research, Leuven, Belgium

79. Mitochondrial Biogenesis and Respiration as Sensitive Indicators for Nucleoside Analog Toxicity.

Yili Xu, Adrian S. Ray, Brian Schultz, Roman Sakowicz, Joy.Y Feng

Gilead Sciences Inc., Foster City, CA, United States

80. A Leading DAA ZN2007 as HCV NS3 Inhibitor with Excellent Activity and Safety by SAR Optimization for Clinical Study.

Zheng-Yun James. Zhan\textsuperscript{1,2}, Guoyan Zhang\textsuperscript{2}, Hua Yan\textsuperscript{1}, Xianjing Yu\textsuperscript{2}

\textsuperscript{1}AB Pharma Ltd., Shanghai, China, \textsuperscript{2}Zannan SciTech Co., Ltd., Shanghai, China
Program Schedule

**Wednesday, May 14, 2014**

**ANTONIN HOLÝ AWARD LECTURE**

**BALLROOM A**

8:30 am – 9:15 am

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8:30 am 81. From Modified Nucleoside to a Chemical Modified Genome.

Piet Herdewijn, Ph.D.
Rega Institute, Belgium

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**MEDICINAL CHEMISTRY**

Chair(s): Chris Meier, M.D.

**BALLROOM A**

9:15 am – 10:15 am

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Michela Cancellieri¹, Marcella Bassetto¹, Gilda Giancotti¹, Pieter Leyssen², Johan Neyts², Andrea Brancale¹
¹Cardiff, Wales, United Kingdom, ²University of Leuven, Belgium

9:30 am 83. Development of the Central Nervous System (CNS)-Targeting Protease Inhibitors for Treating HIV-Associated CNS Disorders.

Hiroaki Mitsuya¹,², Masayuki Amano², Miguel Gómez², Manabu Aoki², Hironori Hayashi², Sofiya Yashchuk³, Debananda Das¹, Arun Ghosh³
¹Exp Retrovirol Sec, Nat’l Cancer Inst, NIH, Bethesda, Maryland, United States, ²Depts of Infect Dis and Hematol, Kumamoto Univ Sch Med, Kumamoto City, Kumamoto, Japan, ³Depts of Chem and Med Chem, Purdue Univ, West Lafayette, Indiana, United States

9:45 am 84. Synthesis and Characterization of Biologically Active Nucleoside Triphosphate Prodrugs.

Tristan Gollnest¹, Jan Balzarini², Chris Meier¹
¹University of Hamburg, Hamburg, Germany, ²Katholieke Universiteit Leuven, Leuven, Flemish Brabant, Belgium

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10:00 am 85. Chemical Optimization of Novel Inhibitor Classes Selectively Targeting PI4KIIIb: a Host Lipid Kinase Crucial for Enterovirus Replication.

J. Brad Shotwell, Shihyun You, Lisa Shewchuk, Liping Wang, Scott Dickerson, Ping Xiong, Rich Peterson, Jeff Gobel
GlaxoSmithKline, Research Triangle Park, NC, United States

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**COFFEE BREAK**

**BALLROOM LOBBY**

10:15 am – 10:45 am
ANTIVIRAL TARGETS AND MECHANISM OF ACTION

Chair(s): Johan Neyts, Ph.D. and Justin Julander, Ph.D.

BALLROOM A

10:45 am – 12:00 pm

10:45 am 86. Benzhydrylpiperazine Compounds Inhibit Cholesterol-Dependent Cellular Entry of Hepatitis C Virus.
Ana M. Chamoun-Emanuelli, Zhilei Chen
Texas A&M University, College Station, TX, United States

10:50 am 87. Identification of an Inhibitor of HIV-1 Vif-Dependent Degradation of Apobec3G.
Erez Pery1,2, Ann Sheehy3, N. Miranda Nebane4, Marie K. Mankowski5, Lynn Rasmussen4, E. Lucile White4, Roger G. Ptak5, Dana Gabuzda1,6
1Dept. Cancer Immunology and AIDS, Dana Farber Cancer Institute, Boston, MA, United States, 2Dept. Pathology, Harvard Medical School, Boston, MA, United States, 3Dept. Biology, College of the Holy Cross, Worcester, MA, United States, 4High Throughput Screening Center, Southern Research Institute, Birmingham, AL, United States, 5Dept. Infectious Disease Research, Southern Research Institute, Frederick, MD, United States, 6Dept. Neurology (Microbiology), Harvard Medical School, Boston, MA, United States

Nidya Segura Guerrero1, Leen Delang1, Ali Tas2, Gilles Querat3, Byron Martina4, Johan Neyts1, Martijn van Hemert2, Pieter Leyssen1
1University of Leuven, Leuven, Belgium, 2Leiden University Medical Center, Leiden, Netherlands, 3UMR_D 190, Aix-Marseille University, Marseille, France, 4Erasmus Medical Center, Rotterdam, Netherlands

11:00 am 89. Immune Modulators are Effective in Reducing Disease in a Mouse Model of Chikungunya.
Justin G. Julander, Ashley Dagley
Institute for Antiviral Research, Utah State University, Logan, UT, United States

11:05 am 90. Targeting Membrane-Bound Viral RNA Synthesis Reveals Potent Inhibition of Diverse Coronaviruses Including the Middle East Respiratory Syndrome Virus.
Anna Lundin1, Ronald Dijkman2,3, Tomas Bergstrom1, Volker Thiel2,3,4, Edward Trybala1
1University of Gothenburg, Gothenburg, Sweden, 2Institute of Immunobiology, Kanontal Hospital, St Gallen, Switzerland, 3Federal Institute of Virology and Immunology, Berne, Switzerland, 4University of Berne, Berne, Switzerland

11:15 am 91. Mechanism of Inhibition for BMS-791325, a Non-Nucleoside Inhibitor of Hepatitis C Virus NS5B Polymerase in Phase 3 Clinical Studies.
Karen Rigat, Hao Lu, Ying-Kai Wang, John Kadow, Min Gao, Lynn Abell, Julie Lemm, Susan Roberts
Bristol-Myers Squibb Co., Wallingford, CT, United States
11:25 am  93. Characterization of MBX2329 and MBX2546- Unique Small Molecule Inhibitors of Influenza A Virus.
   Arnab Basu¹, Gloria Komazin-Meredith¹, Donald T. Moir¹, Dale L. Barnard², Lijun Rong³, Terry L. Bowlin¹
   ¹Microbix Inc, Worcester, MA, United States, ²Utah State University, Logan, UT, United States, ³University of Illinois at Chicago, Chicago, IL, United States

11:35 am  94 The Selectivity of Cidofovir for HPV-Positive Cells is Based on the Differential Response to DNA Damage of Normal Cells and Cancer Cells.
   Graciela Andrei, Tilm De Schuttter, Dimitrios Topalis, Lieve Naesens, Robert Snoeck
   Rega Institute for Medical Research, Leuven, Belgium

   David Olagnier¹, Florine Scholte², Cindy Chiang², Irina Albulescu¹, Rongtuan Lin³, Eric Snijder², John Hiscott¹, Martijn van Hemert²
   ¹Vaccine & Gene Therapy Institute of Florida, Port St. Lucie, FL, United States, ²Leiden University Medical Center, Leiden, ZH, Netherlands, ³McGill University, Montreal, QC, Canada

WEDNESDAY LUNCH (on your own)
12:00 pm – 1:30 pm

ISAR BUSINESS MEETING
   Chair(s): Phil Furman, Ph.D.
   BALLROOM A
   1:30 pm – 2:00 pm

RESEARCH TRIANGLE PARK
   Chair(s): Randal Lanier, Ph.D. and Ronald Swanstrom, Ph.D.
   BALLROOM A
   2:00 pm – 4:30 pm

2:00 pm  96. Biophysical Mechanisms and Methods of Evaluation in HIV Prevention Science.
   David Katz, Ph.D.
   Duke University, USA

   Ralf Baric, Ph.D.
   University of North Carolina, USA
COFFEE BREAK
BALLROOM LOBBY
3:00 pm – 3:30 pm

3:30 pm 98. Did We Put the Cart Before the Horse? Clinical Pharmacology Insights into HIV Prevention Trial Outcomes.
Angela Kashuba, Pharm.D.
University of North Carolina at Chapel Hill, USA

4:00 pm 203. The Novel Nucleoside Analog BCX4430 Exhibits Broad-Spectrum Antiviral Activity and Confers Post-Exposure Protection against Ebola and Marburg Viruses
Travis K. Warren, Ph.D.
USAMRIID

POSTER SESSION II (ABSTRACTS 99-166, 184-202)
BALLROOM C
4:30 pm– 6:00 pm

M. Chandramohan1, D. Sivakumar1, S.C. Vivekananthan1, M. Kannan1, P. Selvam2
1 Kamarajar liver hospital and Research Centre, Madurai, TN, India, 2Nova College of Pharma. Edu and Research, Jupudi, A.P, India

100. The Dengue Virus NS1 Protein Modulates Innate Immune Signaling Early During Infection.
Farah Alayli, Frank Scholle
NC State University, Raleigh, NC, United States

101. Antiviral Nanonase-Tm.
Jacob G. Appelbaum, Rudolf I. Salganik
AVIRID, INC., Gainesville, Florida, United States

102. Unique Features Identify CCR5-Using Macrophage-Tropism as a Rare Phenotype of HIV-1 Distinguishable from CCR5-Using T Cell-Tropism.
Kathryn T. Arrildt1, Sarah B. Joseph1, Celia C. LeBranche2, Ean Spielvogel1, Zaki Dard1, David C. Monetefiori2, Ron I. Swanstrom1
1University of North Carolina, Chapel Hill, NC, United States, 2Duke University, Durham, NC, United States

103. Efficacy of the Beraprost Isomer gp1681 for Treating Influenza Virus A H5N1 Infections in Mice.
Dale Barnard1, Jiing-Huey Lin2, William Guilford2, Daryl Faulds2
1Utah State University, Logan, Utah, United States, 2Gemmus Pharma, San Francisco, California, United States
104. Innate Immune Agonists Demonstrate Pre-Clinical Efficacy and Tolerability Representing a Novel Class of Broad Spectrum Antivirals.
Kristin Bedard¹, Ikenna Madu¹, Shari Kaiser¹, Myra Wang¹, Michael Gale, Jr², Shawn Iadonato¹
¹KINETA, Inc, Seattle, WA, United States, ²University of Washington, Seattle, WA, United States

105. Mode of Action of gp1681 as a Therapeutic for Influenza A Infections.
Daryl Faulds¹, Dale Barnard², William Guilford³
¹Gemmus Pharma Inc, San Francisco, California, United States, ²Utah State University, Logan, Utah, United States

106. Nanoemulsion-Adjuvanted Vaccines Induce Robust Protection Against Genital HSV-2 Infection in a Guinea Pig Model.
R. Cardin¹, F. Bravo¹, T. Hamouda², V. Bitko², C-A. Malinczak², J. Sun², A. Fattom², D. Bernstein¹
¹Cincinnati Children’s Hospital Medical Center, Cincinnati, OH, United States, ²NanoBio Corp., Ann Arbor, MI, United States

Sreerupa Challa, Choi Lai Tiong-Yip, Qin Yu
Infection Innovative Medicines Unit, AstraZeneca R&D Boston, Waltham, MA, United States

108. Study on Inhibitors of the Herpes Simplex Virus Type 1 Alkaline Nuclease.
Tian Chen, Lei Zhang, Hongyu Chen, Kang Cao, Jin Hu, Qu Pan
Chengdu Medical College, Chengdu, Sichuan, China

109. Inhibition of Hsp90 by Small Molecules Cures Human Herpesvirus 8 Persistence.
Wuguo Chen¹, Sang-Hoon Sin², Kwun Wah Wen³, Blossom Damania⁴, Dirk Dittmer⁵
¹University of North Carolina, Chapel Hill, NC, United States, ²University of North Carolina, Chapel Hill, NC, United States, ³University of North Carolina, Chapel Hill, NC, United States, ⁴University of North Carolina, Chapel Hill, NC, United States, ⁵University of North Carolina, Chapel Hill, NC, United States

111. Formulation of Cidofovir Improves the Anti-Papillomaviral Activity of Topical Treatments in the CRPV/Rabbit Model.
Neil D. Christensen, Nancy M. Cladel, Jiafen Hu, Balogh K. Karla
Penn State University, College of Medicine, Hershey, PA, United States

112. A Mouse Papillomavirus Model to Study Anti-Viral Responses to Cutaneous and Anogenital Mucosal Infections.
Neil D. Christensen, Nancy M. Cladel, Lynn R. Budcon, Karla K. Balogh
Penn State University, College of Medicine, Hershey, PA, United States

113. A Small Molecule Inhibitor of Virion Attachment to Heparan Sulfate- or Sialic Acid-Containing Glycans.
Che C. Colpitts, Luis M. Schang
Li Ka Shing Institute of Virology, Edmonton, Alberta, Canada
114. A Combined Cell Based and Site Directed Mutagenesis Approach Defines Highly Conserved Residues Involved in the Selective Inhibition of the HIV-1 Ribonuclease H Function by Diketoacid Derivatives.
Angela Corona¹, Sandro Cosconati², Sylvain Thierry³, Francesco S. Di Leva⁴, Olivier Delelis³, Francesca Esposito¹, Roberto Di Santo⁵, Enzo Tramontano¹
¹University of Cagliari, Cagliari, Italy, ²DISTABiF, Università Napoli 2, Caserta, Italy, ³LBPA, ENS, Cachan, France, ⁴Italian Institute of Tecnology, Genoa, Italy, ⁵La Sapienza University, Rome, Italy

115. Dioxolane L-Nucleoside Analogue, L-BHDU, Inhibits VZV Replication by Depleting the Cellular dTTP Pool.
Chandrav De¹, Uma S Singh², Chung K Chu², Fred Hagen³, Jennifer F Moffat¹
¹SUNY Upstate Medical University, Syracuse, NY, United States, ²University of Georgia, Athens, GA, United States, ³University of Rochester Medical Center, Rochester, NY, United States

Bryan E. Bunnell², Dongmei Liu¹, Jennifer F. Moffat¹
¹SUNY Upstate Medical University, Syracuse, New York, United States, ²Syracuse University, Syracuse, New York, United States

117. Biosynthesis and Degradation of the Triphosphates of Cyclopropavir and Ganciclovir in Human Cytomegalovirus Infected Cells.
Brian G Gentry¹, John C Drach²
¹Drake University College of Pharmacy and Health Sciences, Des Moines, IA, United States, ²University of Michigan School of Dentistry, Ann Arbor, MI, United States

118. The Discovery and In Vitro Characterization of Pyrido-Pyrimidinone Antiretrovirals with Selective Activity Against HIV Ribonuclease H as well as Dual Inhibitors of Both Ribonuclease H and Integrase.
Peter Gerondelis, John W. Seal III, Derek J. Parks, Kendra E. Hightower, Kevin W. Brown, Robert G. Ferris, Emile J. Velthuisen, Brian A. Johns
GlaxoSmithKline, Research Triangle Park, NC, United States

119. STING Agonist Induces a Potent Innate Antiviral Immune Response Against Hepatitis B Virus.
Fang Guo¹, Fei Liu¹, Xuesen Zhao¹,2, Timothy M. Block¹,2, Ju-Tao Guo¹, Jinhong Chang¹
¹Drexel University College of Medicine, Doylestown, PA, United States, ²Baruch S. Blumberg Institute, Hepatitis B Foundation, Doylestown, PA, United States

120. Antiviral Activity of Vemurafenib Against Influenza A Virus in Mice and Cells with Wild Type BRAF- Indication of a New Mode of Action.
Emanuel Haasbach¹, Carmen Hartmayer¹, Stefanie Hildenbrand¹, Stephan Ludwig², Oliver Planz¹
¹University of Tuebingen, Tuebingen, Germany, ²University of Muenster, Muenster, Germany

121. Reserve Autophagic Capacity in Alveolar Epithelia Provides a Replicative Niche for Influenza A Virus.
David R Hahn, Cheng-Lun Na, Timothy E Weaver
Perinatal Institute, Section of Neonatology, Perinatal and Pulmonary Biology Cincinnati Children’s Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, OH, United States
122. **RASD1: A Novel Gene Target of HSV-2.**
   Susan C. Irvin¹, Natalia Cheshenko¹, Viviana Simon², Betsy C. Herold¹
   ¹Albert Einstein College of Medicine, Bronx, NY, United States, ²Mount Sinai School of Medicine, New York, NY, United States

123. **SB 9200, a Novel Anti-HBV Agent—In Vitro Combination Studies and Pharmacodynamic Studies in Woodchucks.**
   R.P. Iyer¹, A. Sheri¹, R.K. Pandey¹, S. Padmanabhan¹, J.K. Marquis¹, J.M. Skell¹, B.E. Korba², J.D. Morrey³
   ¹Spring Bank Pharmaceuticals, Milford, MA, United States, ²Georgetown University Medical Center, Washington, DC, United States, ³Institute for Antiviral Research, Utah State University, Logan, UT, United States

124. **Genetic Vaccine Constructed with Hantavirus Gn Targeting to MIIC by Lysosome-Associated Membrane Protein, Conferred Balb/C Mice Satisfying Immune Protection Against HTNV Infection.**
   Dongbo Jiang, Yuanjie Sun, Linfeng Chen, Gefei Zhang, Fanglin Zhang, Kun Yang
   Fourth Military Medical University, Xi’an, Shaanxi, China

125. **Efficiency of Incorporation and Chain Termination Determines the Inhibition Potency of 2’-Modified Nucleotide Analogs Against HCV Polymerase.**
   Zhinan Jin, Amy Fung, Natalia Dyatkina, Guangyi Wang, Leo Beigelman, Jerome Deval
   Alios BioPharma Inc., South San Francisco, CA, United States

126. **Down Regulation of M2 Gene and Inhibition of Influenza Virus Replication in Host Cells Using Catalytic Nucleic Acid Enzymes.**
   Madhu Khanna, Binod Kumar, Roopali Rajput, Latika Saxena
   Department of Respiratory Virology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

127. **Inhibition of Influenza B Virus M1 Protein by 3H,3’H-Spiro[Benzofuran-2, 1’-Isobenzofuran]-3,3’-Dione.**
   Meehyein Kim, Ye Jin Jang, Yun Young Go, Chonsaeng Kim, Yashwardha Malpani, Young-Sik Jung, Chong-Kyo Lee
   Division of Drug Discovery Research, Korea Research Institute of Chemical Technology, Daejeon, Daejeon, South Korea

128. **Structure-Based Drug Design of Novel Active Site and Allosteric HIV-1 Rnase H Inhibitors.**
   Karen A. Kirby¹,², Hilary A. Schmidt¹,², Jing Tang³, Tatiana Ilnia⁴, Qiongying Yang¹,², Zhengqiang Wang³, Michael A. Pariniak⁴, Stefan G. Sarafianos¹,²,⁵
   ¹Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO, United States, ²Department of Molecular Microbiology & Immunology, University of Missouri School of Medicine, Columbia, MO, United States, ³Center for Drug Design, University of Minnesota, Minneapolis, MN, United States, ⁴Department of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, United States, ⁵Department of Biochemistry, University of Missouri, Columbia, MO, United States
129. MAPKAP Kinase 3 (MK3) Suppresses IFN-Gamma Gene Expression and Attenuates NK Cell Cytotoxicity and TH1 CD4 T Cell Development in Influenza A Virus Infected Mice.
Katharina Koether¹, Carolin Nordhoff¹, Jay H. Bream², Matthias Gaestel³, Viktor Wixler¹, Stephan Ludwig¹
¹Institute of Molecular Virology, Muenster, NRW, Germany, ²Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, ³Hannover Medical School, Hannover, Lower-Saxonia, Germany

Rajesh Kumar¹, Ruchi Kumari¹, Raiees Andrabi¹, Ashutosh Tiwari¹,³, Hilal Ahmed¹, Lubina Khan¹, Subrata Sinha¹,², Kalpana Luthra¹
¹Department of Biochemistry, New Delhi, India, ²National Brain Research Centre, Manesar, Harayana, India, ³Translational Health Science and Technology Institute (THSTI), Gurgaon, Harayana, India

131. Fast HCV RNA Elimination and NS5A Redistribution by Daclatasvir.
Dandan Liu¹, Juan Ji¹, Tanya P. Ndongwe¹, Charles M. Rice², Robert O. Ralston¹, Stefan G. Sarafianos¹
¹University of Missouri-Columbia, Columbia, Missouri, United States, ²The Rockefeller University, New York, New York, United States

Duncan Matheka, Jolynne Mokaya, Marybeth Maritim
University of Nairobi, Nairobi, Kenya

133. Expression of Immunological Markers in Liver Tissue of HIV/HCV Co-Infected Patients.
Natallia V. Matsuieuskaya, Vladimir M. Tsyrkunov, Michail G. Zubritskiy, Nikolay I. Prokopchik
Grodno State Medical University, Grodno, Belarus

Paul L. Maurizio, Martin T. Ferris, Alan C. Whitmore, William Valdar, Mark T. Heise
University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

135. The Impact of the IEC (Information, Education and Communication) in the Affidavit of Sex Workers: Study Realized by the Youth Center Coulibaly Sidiki of the University of Kinshasa (Democratic Republic of Congo).
Floribert M. MONGA¹, Gaetan M. MUTOMBO²
¹Free University of Kinshasa, Kinshasa, Democratic Républic of Congo, Congo-Kinshasa, ²Youth Center Coulibaly Sidiki, Kinshasa, Democratic Republic of Congo, Congo-Kinshasa

136. A Versatile In Vitro Assay Identifies Inhibitors and Stimulators of Nonsegmented Negative-Sense RNA Virus Polymerase Function.
Benjamin Morin¹, Linda J. Rennick², W. Paul Duprex², Sean P. J. Whelan¹
¹Harvard Medical School, Boston, MA, United States, ²Boston University School of Medicine, Boston, MA, United States
137. **Optogenetic Approaches for Measuring Motor Function Deficits in Arboviral Encephalitis.**
John D. Morrey, Hong Wang, Venkatraman Siddharthan, Neil E. Motter, Joseph W. Clyde, Justin G. Julander
Utah State University, Logan, UT, United States

138. **Antiretroviral Therapy (ART) May Reduce the Elevated Levels of Tumor Marker in HIV/AIDs Patients.**
Saif Ullah Munshi, Afsana Miti, Nahida Sultana, S M Rashed Islam, Shahina Tabassum
Bangabandhu Sheikh Mujib Medical University, Dhaka, Dhaka, Bangladesh

139. **Entecavir Reduces Viral Load and Hepatic Injury in Chronic Hepatitis B But Fails to Normalize Immunological Changes.**
Saif Ullah Munshi, Nusrat Sultana, Manzurl Haque, Shahina Tabassum
Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Dhaka, Bangladesh

140. **P-Body Component MOV10 Inhibits HCV Virus Production and Infectivity.**
Tanyaradzwa Ndongwe¹,², Robert Ralston¹,²,³,⁴, Taisuke Izumi⁵, Vinay Pathak⁶,
Stefan Sarafianos¹,²,⁶
¹Christopher Bond Life Sciences Center, Columbia, Missouri, United States,
²Department of Molecular Microbiology & Immunology, University of Missouri, School of Medicine,
³Columbia, Missouri, United States,
⁴Liver Center, University of Kansas Medical Center,
⁵Viral Mutation Section, HIV Drug Resistance Program, National Cancer Institute-Frederick, Frederick, Maryland, United States,
⁶Department of Biochemistry, University of Missouri, Columbia, Missouri, United States

141. **Galectin-3 Interacts with HIV-1 Tat in Latently Infected Cells.**
Mika Okamoto, Akemi Hidaka, Masaaki Toyama, Masanori Baba
Kagoshima University, Kagoshima, Japan

142. **Discovery of a Small Molecule Compound Series with Potent Activity Against Hepatitis C Virus NS4B by Screening Using Encoded Library Technology.**
Michael Thomson¹, Zhengrong Zhu², Hamilton Dickson¹, Derek Parks³, Jesse Keicher¹,
Ken Lind², Randy Bledsoe³, Christopher Arico-Muendel²
¹AV DPU, GlaxoSmithKline, RTP, NC, United States,
²ELT Boston, GlaxoSmithKline, Boston, MA, United States,
³Biological Sciences, GlaxoSmithKline, RTP, NC, United States

143. **Control of SAMHD1 Mediated Restriction of HIV-1 Replication.**
Eduardo Pauls, Roger Badia, Marc Permanyer, Eva Riveira-Muñoz, Bonaventura Clotet,
Ester Ballana, Jose Este
AIDS Research Institute – IrsiCaixa, Badalona, Barcelona, Spain

144. **Protective Efficacy of Novel mRNA Vaccines Against Influenza Virus Infection.**
B. Petsch¹, M. Schnee¹, A. Vogel², D. Voss¹, K.-J. Kallen¹, L. Stitz², T. Kramps¹,³
¹CureVac GmbH, Tübingen, BW, Germany,
²Friedrich-Loeffler-Institut, Institute of Immunology, Greifswald, MV, Germany,
³present adress: Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, H, Germany,
⁴present adress: Roche Pharma AG, Grenzach-Wyhlen, BW, Germany
Koreen Ramessar1, Chang Yun Xiong1, Lauren R.H. Krumpe2, Raymond C. Sowder II3,
Robert W. Buckheit Jr.4, James B. McMahon1, Barry R. O’Keefe1
1Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute,
Frederick, MD, United States, 2Molecular targets Laboratory, Leidos Inc., Frederick, MD, United States, 3AIDS and Cancer Virus Program, Leidos Inc., Frederick, MD, United States, 4Imquest BioSciences Inc., Frederick, MD, United States

146. Anti-HSV-2 Activity of the Glycoconjugate PG545 in a Mouse Model of Genital Herpes Infection.
Joanna S. Said1, Edward Trybala1, Eva Jennische2, Stefan Lange3, Staffan Görander1,
Maria Ekblad1, Jan-Åke Liljeqvist1, Tomas Bergström1
1Department of Clinical Virology, University of Gothenburg, Goteborg, Vastra Gotaland, Sweden, 2Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Goteborg, Vastra Gotaland, Sweden, 3Department of Clinical Bacteriology, University of Gothenburg, Goteborg, Vastra Gotaland, Sweden

147. Enhancing Clinical Competency At Every Angle-ViroChannel as a New Model for Clinical Education.
M Selbovitz1, D Miller1, D Lecavalier2
1Cornell ACTG, New York, NY, United States, 2ViroChannel, Montreal, Canada

148. Design and Synthesis of Novel Fluoroquinolones as Potential Inhibitors of HIV Integrase.
Periyasamy Selvam1, M Kathur Reddy2, Yves Pommier2, Christophe Marchand2
1Nova College of Pharmaceutical Education and Research, Ibrahimpatnam, Krishna Dt, Andrapradesh, India, 2Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, Bethesda, Maryland, United States

149. LEDGF- HIV Integrase Inhibitory Activity of 2-Methylpyrazole and 2-Amino-4-Phenyl-Thiazole.
Periyasamy Selvam1, Guoping Hu2, Yun Tang2, Xi Li2, Jin Huang2
1Nova College of Pharmaceutical Education and Research, Jupudi, A.P, India, 2School of Pharmacy, East China University of Science and Technology, Shanghai 200237, Shanghai, China

150. Investigation of Anti-HIV Activity and Cytotoxicity of Morinda Citrifolia Noni Fruit Extracts.
Periyasamy Selvam1, T Paul Pandi1, Christophe Panecouque2, Erik De Clercq2
1Nova college of Pharmaceutical Education and Research, Jupudi, A.P, India, 2Rega Institute for Medical Research, Leuven, Flander, Belgium

151. Design, Synthesis and Molecular Modelling Studies Of Quinazolin-3(4H)-One Derivatives As Novel Inhibitors of HIV Integrase.
Periyasamy Selvam1, Nouri Neamati2, Tino Sanchez2
1Nova College of Pharmaceutical Education and Research, Jupudi, Krishna Dt, Andrapradesh, India, 2Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, CA, United States
152. **Brincidofovir (BCV, CMX001) Delivers High Intracellular Concentrations of Cidofovir Diphosphate.**  
*Phiroze Sethna, Dean Selleseth, Andrew Bae, Laurie Keilholz, Bernhard Lampert, Randall Lanier*  
*Chimerix Inc, Durham, NC, United States*

153. **Nucleic Acid Scavengers: Impact on Inflammatory Disorders and Viral Susceptibility.**  
*Kara L. Shumansky, Eda K. Holl, Angelo Moreno, George A. Pitoc, Elizabeth Ramsburg, Bruce A. Sullenger*  
*Duke University Medical Center, Durham, NC, United States*

154. **Targeting an Immunomodulatory West Nile Virus Protein to Improve Vaccine Candidate Efficiency.**  
*Lindsey Stevenson, Frank Scholle*  
*North Carolina State University, Raleigh, NC, United States*

155. **Effect of a Triple Anti-Enteroviral Combination Applied in Consecutive Alternative Administration (CAA) Course in Coxsackievirus B1 Neuroinfection in Mice.**  
*A. Stoyanova, I. Nikolova, A. S. Galabov*  
*The Stephan Angeloff Institute of Microbiology, Bulg. Acad.Sci., Sofia, Bulgaria*

156. **Abalone Hemocyanin Inhibits Herpes Simplex Virus Type 1 Infection In Vitro.**  
*Negar Talaei Zanjani, Monica Miranda Saksena, Anthony L. Cunningham, Peter Valtchev, Vincent G. Gomes, Fariba Dehghani*  
*1School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW, Australia, 2Centre for Virus Research, Westmead Millennium Institute, Sydney, NSW, Australia*

157. **Anti-Influenza and Anti-Inflammatory Activity of KPT-335, a Selective Inhibitor of Nuclear Export (SINE), in Mice and Ferrets.**  
*Sharon Tamir, Olivia Perwitasari, Scott K. Johnson, Yosef Landesman, Joel Ellis, Sharon Shacham, Robert O. Carlson, Ralph A. Tripp*  
*1Department of Infectious Diseases, Animal Health Research Center, University of Georgia, Athens, GA, United States, 2Karyopharm Therapeutics, Natick, MA, United States*

158. **Pharmacokinetics and Pharmacodynamics of Tenofovir and Tenofovir Disoproxil Fumarate in the Female Genital Tract.**  
*Ekaterina S. Taneva, Leslie A. Geer, Pedro M.M. Mesquita, Betsy C. Herold*  
*1Albert Einstein College of Medicine, Bronx, New York, United States, 2Particle Sciences, Bethlehem, PA, United States*

159. **Weight Loss Cutoff for Mortality in Mice Impacts the Results from Combination Drug Therapy for an Influenza A (H1N1) Virus Infection.**  
*Bart Tarbet, Deanna Larson, Min-Hui Wong, Donald Smee*  
*Utah State University, Logan, Utah, United States*

160. **Potential of Novel Acylguanidine-Based Small Molecules with Broad-Spectrum Viroporin Activity as Dual Inhibitors of HIV-1 and Hepatitis C Virus.**  
*Ian Tietjen, Philip Mwimanzi, Scott C Miller, Aniqa Shahid, Zabrina L Brumme, Mark A Brockman, David Fedida*  
*1University of British Columbia, Vancouver, British Columbia, Canada, 2Simon Fraser University, Burnaby, British Columbia, Canada*
161. Characterization of a Novel Respiratory Syncytial Virus Inhibitor.
  Choi-Lai Tiong-Yip, Lisa Aschenbrenner, Kenneth D. Johnson, Robert E. McLaughlin, Jun Fan, 
  SreeRupa Challa, Hui Xiong, Qin Yu 
  AstraZeneca Infection iMed, Boston, MA, United States

162. NGS Nominated Genes for Predisposition to Balkan Endemic Nephropathy (BEN).
  D. Toncheva 
  Department of Medical Genetics, Medical University 2Genomics Laboratory, Malinov Clinic, 
  Sofia, Bulgaria

163. Identification of Novel Inhibitors of HBV Replication by In Silico Screening Targeting Capsid Assembly.
  Masaaki Toyama¹, Takayuki Hamasaki¹, Mika Okamoto¹, Koichi Watashi², Takaji Wakita², 
  Masanori Baba¹ 
  ¹Kagoshima University, Kagoshima, Kagoshima, Japan, ²National Institute of Infectious Diseases, Shinjuku-ku, Tokyo, Japan

  Lonneke van der Linden¹,², Laia Vives-Adrián³, Barbara Selisko⁴, Bruno Coutard⁴, 
  Gerhard Puerstinger⁵, Nuria Verdaguer³, Johan Neyts², Frank van Kuppeveld⁶ 
  ¹Dept Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, 
  Netherlands, ²Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium, ³Institut de Biologia Molecular de Barcelona (CSIC), Barcelona, 
  Spain, ⁴Laboratoire d'Architecture et Fonction des Macromolécules Biologiques, CNRS, 
  University Marseille, Marseille, France, ⁵Dept Pharmaceutical Chemistry, Institute of Pharmacy, 
  University of Innsbruck, Innsbruck, Austria, ⁶Dept Infectious Diseases and Immunology, Utrecht 
  University, Utrecht, Netherlands

165. Discovery of a Small Molecule Triggering Innate Immunity.
  Shihyun You, Anna Banka, Hamilton Dickerson, Margaret Gartland, Cindy Richards, 
  J.Brad Shotwell, Michael Thomson, Mi Xie 
  GlaxoSmithKline, RTP, NC, United States

166. Regulation and Function of Efflux Transporters in Mouse Cervicovaginal Tissues.
  Tian Zhou¹,², Minlu Hu¹,², Andrew Pearlman², Lisa Rohan¹,² 
  ¹Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, 
  Pittsburgh, PA, United States, ²2Magee-Womens Research Institute, Pittsburgh, PA, 
  United States

185. Identification of Host Factors Involved in Lipid Droplet Homeostasis and the Replication of Hepatitis C and Dengue Virus by RNAi Screening
  Ina Karen Stoewen¹, Gualtiero Alvisi², Sandeep Amberkar³, Narsis Kiani³, Christoph Sommer⁴, 
  Wolfgang Fischl¹, Marion Poenisch¹, Fred A. Hamprecht⁴, Giorgio Palù², Lars Kaderali³, 
  Ralf Bartenschlager¹ 
  ¹Department of Infectious Diseases, Heidelberg University, Heidelberg, 
  Germany, ²Department of Molecular Medicine, University of Padua, Padua, Italy, ³Institute 
  of Medical Informatics & Biometry, Dresden University of Technology, Dresden, 
  Germany, ⁴Heidelberg Collaboratory for Imaging Processing, Heidelberg University, 
  Heidelberg, Germany
187. Evaluation of Trans-complementation for Dengue Virus Serotype 2 Non Structural Protein 4B, a Target For Inhibition of Flavivirus Replication
Ilane Hernandez-Morales¹, Marnix Van Loock¹, Kai Dallmeier², Johan Neyts², Gregory Fanning¹
¹Janssen Infectious Diseases BVBA, Beerse, Belgium, ²Rega Institute, KU Leuven
(#Equal contribution), Leuven, Belgium

188. The Chikungunya Virus Replication Complex: In Vitro Characterization and Mode of Action Studies on Antiviral Compounds
Irina Albulescu, Ali Tas, Florine Scholte, Eric Snijder, Martijn van Hemert
Leiden University Medical Center, Leiden, Netherlands

189. Investigation of Calicivirus Replication Complex Formation
Yuka Otsuka¹, Charlotte Melia², Montserrat Bárcena², Jacques Rohayem¹,³
¹Riboxx GmbH, D-01445, Radebeul, Germany, ²Leiden University Medical Center, Leiden, Netherlands, ³Institute of Virology, Medical Faculty, Technische Universität Dresden, Germany

190. Host Cell Cholesterol Landscape Is Important for Efficient Picornavirus Replication
Lucian Albulescu, Jeroen RPM Strating, Frank JM van Kuppeveld
Division of Virology, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht, Netherlands

191. Development of Suitable Surrogate Models to Investigate the Mechanism of Action of Novel FMDV Inhibitors
Denny Kollanur¹, Lyre Espada Murao¹, Jan Swinnen¹, Annebel R De Vleeschauwer², David J Lefebvre², Kris De Clercq², Johan Neyts¹,³, Nesya Goris¹
¹Okapi Sciences NV, Heverlee, Belgium, ²Veterinary and Agrochemical Research Center (CODA-CERVA), Brussels, Belgium, ³Rega Institute for Medical Research, University of Leuven (KU Leuven), Leuven, Belgium

192. Mapping of RNA Structural Elements in the Sapovirus Genome
Subash K. Rai¹, Alexander P. Gultyaev², Alexander E. Gorbalenya³,⁴, Jacques Rohayem¹,⁵
¹Riboxx GmbH, Pharmapark, Radebeul, Germany, ²Dept. of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands, ³Dept. of Med. Microbiology, Leiden University Medical Center, Leiden, Netherlands, ⁴Faculty of Bioengineering & Bioinformatics, Lomonosov Moscow State University, Moscow, Russia, ⁵Institute of Virology, Medical Faculty, Technische Universität Dresden, Dresden, Germany

193. Shape-based Virtual Screening for the Identification Of Novel DENV Helicase Inhibitors.
Iuni M. L. Trist¹, Suzanne Kaptein², Pieter Leyssen², Channing Li³, Johan Neyts², Bruno Coutard³, Andrea Brancale¹
¹Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, ²Rega Institute of Medical Research, KU Leuven, Leuven, Belgium, ³AFMB, Aix Marseille Université, Marseille, France

194. Distinct Picornaviruses Rely on Distinct PI4Ks for Replication
Cristina Dorobantu, Hilde van der Schaar, Frank van Kuppeveld
Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Virology Division, Utrecht, Netherlands
Changqing Li, Jaime Guillén, Julie Lichièrè, Bruno Canard, Etienne Decroly, Bruno Coutard
Centre National de la Recherche Scientifique, Aix-Marseille Université, CNRS UMR 7257, AFMB, Marseille, France

196. The Conserved N-terminal Domain Associated with the Nidovirus RNA Polymerase Contains Residues Essential for its Nucleotidylation
Kathleen C Lehmann1, George M C Janssen2, Peter A van Veelen2, Eric J Snijder1, Alexander E Gorbalenya1,2, Clara C Posthuma1
1Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands, 2Department of Immunohematology and Blood Transfusion, Leiden University

197. Proofreading and Ribavirin-5’-monophosphate Excision by SARS-CoV Polymerase/exonuclease Complex
Lorenzo Subissi, Etienne Decroly, Isabelle Imbert, Bruno Canard
AFMB UMR7257 CNRS and Aix-Marseille University, Marseille, France

198. Host-Pathogen Interaction as a Potential Target for Development of Antivirals: Role of SUMOylation in DENV Life Cycle.
Joanna J. Zmurko, Johan Neyts, Kai H. Dallmeier
KU Leuven, Leuven, Belgium

199. A Coxsackievirus Mutant Facilitates an Alternative Site for Replication Under PI4KIIIβ Inhibition
Charlotte Melia1, Hilde M. van der Schaar2, Ronald W. A. L. Limpens1, Eric J. Snijder1, Abraham J. Koster1, Frank J. M. van Kuppeveld2, Montserrat Bárcena1
1Leiden University Medical Center, Leiden, Netherlands, 2University of Utrecht, Utrecht, Netherlands

200. Comparative Study of the Anti-enterovirus 71 Activity of a Selected Series of Enterovirus Inhibitors
Aloys Tijsma1, David Franco1, Rolf Hilgenfeld2, Mathy Froeyen1, Johan Neyts1
1Rega Institute for Medical Research, Leuven, Belgium, 2University of Lübeck, Lübeck, Germany

201. Characterization of the Mode-of-action of a Potent Dengue Virus Capsid Inhibitor
Pietro Scaturro1, Iuni Trist2, David Paul1, Chelsea M. Byrd3, Robert Jordan1, Andrea Brancale2, Ralf Bartenschlager1,4
1Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany, 2School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, 3SIGA Technologies, Inc., Corvallis, USA, 4German Center for Infection Research, Heidelberg, Germany

Ayan K. Chakrabarti1, Brian H. Bird1, Clifton P. Drew2, Ute Stroher1, Stuart T. Nichol1, Christina F. Spiropoulou1
1Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States, 2Infectious Disease Pathology Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States
**Thursday, May 15, 2014**

**WILLIAM PRUSOFF YOUNG INVESTIGATOR AWARD LECTURE**

*Chair(s):* Phil Furman, Ph.D.

**BALLROOM A**

**8:30 am – 9:15 am**

167. Use of Nucleotide Prodrugs to Enhance Selectivity of Anti-HIV and -HCV Agents.

Adrian Ray, Ph.D.

Gilead Sciences, USA

**CHALLENGES IN HIV INFECTION, TREATMENT AND PREVENTION**

*Chair(s):* José Esté, Ph.D.

**BALLROOM A**

**9:15 am – 12:00 pm**

9:15 am 168. Can We Cure HIV Infection.

Mario Stevenson, Ph.D.

University of Miami, USA

9:45 am 169. Potential Therapeutic Approaches for the Cure of HIV Infection.

Derek Sloan, M.D.

Gilead Sciences, USA

**COFFEE BREAK**

**BALLROOM LOBBY**

**10:15 am – 10:45 am**


Gerardo Garcia-Lerma, Ph.D.

Centers for Disease Control and Prevention, USA

11:15 am 171. Monitoring HIV Drugs and Viral Reservoirs.

Courtney Fletcher, Pharm.D.

University of Nebraska, USA

**THURSDAY LUNCH (on your own)**

**12:00 pm – 1:30 pm**
ANIMAL MODELS OF INFECTION
Chair(s): Don Smee, Ph.D., Ramya Natarajan, Ph.D.

BALLROOM A
1:30 pm – 2:30 pm

1:30 pm 172. Preclinical Studies of SB 9200 as an Antiviral Agent Against HBV and HCV.
R.P. Iyer1, B.E. Korba2, R.K. Pandey1, S. Padmanabhan1, J.K. Marquis1, J.M. Skell1, M.L. Harter3
1Spring Bank Pharmaceuticals, Milford, MA, United States, 2Georgetown University Medical Center, Washington, DC, United States, 3MPI Research, Mattawan, MI, United States

1:45 pm 173. Prophylaxis with the Viral Polymerase Inhibitor 2'-C-Methylcytidine Successfully Prevents Transmission of Murine Norovirus from Infected to Uninfected Mice.
Joana Rocha-Pereira, Dirk Jochmans, Johan Neyts
KU Leuven – University of Leuven, Rega Institute for Medical Research, Leuven, Belgium

2:00 pm 174. Combination Therapy of Vaccinia Virus Infections in Immunosuppressed Mice Using Vaccinia Immune Globulin, Parenteral Cidofovir, and Topical Cidofovir.
Donald F. Smee, Ashley Dagley, Brittney Downs, Brett L. Hurst
Utah State University, Logan, Utah, United States

2:10 pm 110. Development of an HIV-Infected, Humanized Mouse Model with Antiviral Pharmacokinetic/Pharmacodynamic Capabilities.
Milloni Chhabra, Jerry Jeffrey, Sonia Miranda, Angela Mote, Barbara Denton, Paula Gardner, Joe Watson, My-Nga Nguyen
GlaxoSmithKline, RTP, NC, United States

2:20 pm 176. A Mouse Model for a Bat Coronavirus HKU5 Variant, a Subgroup 2C Beta Coronavirus.
Sudhakar Agnihotram1, Boyd Yount 1, Eric Donaldson1, Andrew Mesecar2, Arun Gosh2, Mark Denison3, Mark Heise4, Ralph Baric 1
1Departments of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States, 2Department of Medicinal Chemistry, Purdue University, West Lafayette, Indiana, United States, 3Departments of Pediatrics and Pathology and Microbiology and Immunology, Vanderbilt University, Nashville, Tennessee, United States

COFFEE BREAK
BALLROOM LOBBY
2:30 pm – 3:00 pm
CLINICAL EVALUATION OF ANTIVIRAL THERAPIES
Chair(s): David Bernstein, M.D.
BALLROOM A
3:00 pm – 4:00 pm

3:00 pm 177. AVI-7288 Provides Significant Survival Benefit for Marburg Virus Infection.
Patrick L Iversen1,2, Alison Heald1, Travis K Warren3, Jay Charleston1, Peter Sazani1,
Amy C Shurtleff3, Lisa Welch3, Sina Bavari3
1Sarepta Therapeutics, Cambridge, MA, United States, 2Oregon State University, Corvallis,
OR, United States, 3USAMRIID, Fredrick, MD, United States

Olufunmilayo G. Oyero
University of Ibadan, Ibadan, Oyo, Nigeria

3:30 pm 179. Treatment of Influenza Patients with Aprotinin Aerosol Generated by Stationary and Metered Dose Manual Inhalers.
Oleg P. Zhirnov1, Natasha O. Bokova2, Elena I. Isaeva1, Irina V. Vorobjeva1,
Olga A. Saphonova2, Nikolai A. Malyshev2
1The D.I.Ivanovsky Institute of Virology, Moscow, Moscow 123098, Russia,
21'st Moscow Infectious clinics, Moscow, 125367, Russia

3:40 pm 180. IND-Directed Pharmacology and Toxicology of IQP-0528, a Novel HIV-1 Topical Microbicide.
Christian Furlan-Freguia, Karen W. Buckheit, Anthony Ham, Robert W. Buckheit
ImQuest Biosciences, Frederick, MD, United States

3:50 pm 181. Epidemiological and Economic Modelling Study Demonstrating the Potential of Antiviral Agents to Control Classical Swine Fever Outbreaks.
Robert Vrancken1, Jantien Backer2, Johan Neyts1,3, Nesya Goris1
1Okapi Sciences NV, Heverlee, Belgium, 2Central Veterinary Institute of Wageningen UR,
Lelystad, Netherlands, 3Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

SHOTGUN PRESENTATIONS
Chair(s): Katherine Seley-Radtke, Ph.D.
BALLROOM A
4:00 pm – 4:30 pm

CLOSING RECEPTION
BALLROOM LOBBY
7:00 pm – 7:30 pm

CLOSING BANQUET
BALLROOM A
7:30 pm – 10:00 pm
Friday, May 16, 2014

EUVIRNA
Chair(s): Andrea Brancale, Ph.D.
ROOM 304
8:30 am – 12:00 pm

8:30 am 203. Translating Basic Insights in Enterovirus Replication in Antiviral Drug Development.
Frank JM van Kuppeveld, Ph.D.
University of Utrecht, The Netherlands

8:45 am 204. Introduction to Euvirna.
Frank JM van Kuppeveld, Ph.D.
University of Utrecht, The Netherlands

9:00 am 205. In Silico Design of Novel Inhibitors of Picornavirus Replication.
Michela Cancellieri1, Pieter Leyssen2, Rachel Ulferts3, Frank van Kuppeveld3, Johan Neyts2, Andrea Brancale1
1School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom; 2Rega Institute for Medical Research University, Leuven, Belgium; 3Department of Infectious Diseases & Immunology, Utrecht University, Utrecht, The Netherlands.

9:10 am 206. Identification of Host Factors Involved in Lipid Droplet Homeostasis and the Replication of Hepatitis C and Dengue Virus by RNAi Screening.
Ina Karen Stoeck1, Gualtiero Alvisi1,2, Sandeep Amberkar3, Narsis Kiani3, Christoph Sommer4, Wolfgang Fischl1, Marion Poenisch1, Fred A. Hamprecht4, Giorgio Palù3, Lars Kaderali3, Ralf Bartensschlager1
1Department of Infectious Diseases, Molecular Virology, University of Heidelberg, Heidelberg, Germany, 2Department of Molecular Medicine, University of Padua, Padua, Italy, 3Institute of Medical Informatics & Biometry, Dresden University of Technology, Dresden, Germany, 4Heidelberg Collaboratory for Image Processing, University of Heidelberg, Heidelberg, Germany.

Hendrik Jan Thibaut1,6, Lonneke van der Linden1,2, Ping Jiang3, Bert Thys4, María-Dolores Canela5, Leire Aguado5, Bart Rombaut4, Eckard Wimmer3, Aniko Paul3, María-Jesús Pérez-Pérez5, Frank J.M. van Kuppeveld2,6 and Johan Neyts1
1Department of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium, 2Department Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen Centre for Molecular Life Sciences, 6525 HP Nijmegen, The Netherlands, 3Department of Molecular Genetics and Microbiology, School of Medicine, Stony Brook University, 11794 Stony Brook, New York, USA, 4Department of Pharmaceutical Biotechnology & Molecular Biology, Vrije Universiteit Brussel, B-1090 Brussel, Belgium, 5Instituto de Química Médica (IQM-CSIC), 28006 Madrid, Spain, 6Virology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, 3584 CL Utrecht, The Netherlands
9:30 a.m. 208. Dengue Virus Replication and its Implication in Host Interactions.
Ilane Hernandez-Morales¹, Marnix Van Loock¹, Kai Dallmeier²#, Johan Neyts²#,
Gregory Fanning¹
¹Janssen Infectious Diseases BVBA, Beerse, Antwerp, Belgium, ²Rega Institute,
KU Leuven (#Equal contribution), Leuven, Vlaams-Brabant, Belgium

9:40 a.m. 209. The Chikungunya Virus Replication Complex: In Vitro Characterization
and Mode of Action Studies on Antiviral Compounds.
Irina Albulescu, Ali Tas, Florine Scholte, Eric Snijder, Martijn van Hemert
Leiden University Medical Center, Leiden, ZH, Netherlands

Yuka Otsuka¹, Charlotte Melia², Montserrat Bárcena², Jacques Rohayem¹,³
¹Riboxx GmbH, D-01445, Radebeul, Germany, ²Leiden University Medical Center, Leiden,
Netherlands, ³Institute of Virology, Medical Faculty, Technische Universität Dresden, Germany

COFFEE BREAK
ROOM 304 LOBBY
10:00 am – 10:30 am

10:30 am 211. Host Cell Cholesterol Landscape Is Important for Efficient Picornavirus
Replication.
Lucian Albulescu, Jeroen RPM Strating, Frank JM van Kuppeveld
Division of Virology, Department of Infectious Diseases & Immunology, Faculty of Veterinary
Medicine, Utrecht, Utrecht, Netherlands

10:38 am 212. Development of Suitable Surrogate Models to Investigate the Mechanism
of Action of Novel FMDV Inhibitors.
Denny Kollanur¹, Lyre Espada Murao¹, Jan Swinnen¹, Annebel R De Vleeschauwer²,
David J Lefebvre², Kris De Clercq², Johan Neyts¹,³, Nesya Goris¹
¹Okapi Sciences NV, Herleveer, Belgium, ²Veterinary and Agrochemical Research Center
(CODA-CERVA), Brussels, Belgium, ³Rega Institute for Medical Research, University of Leuven
(KU Leuven), Leuven, Belgium

Subash K. Rai¹, Alexander P. Gultyaev², Alexander E. Gorbalenya³,⁴, Jacques Rohayem¹,⁵
¹Riboxx GmbH, Pharmapark, Radebeul, Germany, ²Dept. of Viroscience, Erasmus Medical
Center, Rotterdam, Netherlands, ³Dept. of Med. Microbiology, Leiden University Medical Center,
Leiden, Netherlands, ⁴Faculty of Bioengineering & Bioinformatics, Lomonosov
Moscow State University, Moscow, Russia, ⁵Institute of Virology, Medical Faculty, Technische
Universität Dresden, Dresden, Germany

Iuni M. L. Trist¹, Suzanne Kaptein², Pieter Leyssen², Chanqing Li², Johan Neyts²,
Bruno Coutard², Andrea Brancale¹
¹Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales,
United Kingdom, ²Rega Institute of Medical Research, KU Leuven, Leuven, Belgium, ³AFMB,
Aix Marseille Université, Marseille, France
11:02 am 215. Distinct Picornaviruses Rely on Distinct PI4Ks for Replication.  
Cristina Dorobantu, Hilde van der Schaar, Frank van Kuppeveld  
Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Virology Division, Utrecht, Utrecht, Netherlands

11:10 am 216. Venezuelan Equine Encephalitis Virus NSP1: from Functional Characterization to Understanding of Antiviral Mechanism of Action.  
Changqing Li, Jaime Guillén, Julie Lichière, Bruno Canard, Etienne Decroly, Bruno Coutard  
Centre National de la Recherche Scientifique, Aix-Marseille Université, CNRS UMR 7257, AFMB, Marseille, CEDEX09, France

Kathleen C Lehmann¹, George M C Janssen², Peter A van Veelen³, Eric J Snijder¹, Alexander E Gorbalenya¹³, Clara C Posthuma¹  
¹Department of Medical Microbiology, Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, ²Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands, ³Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Moscow, Russia

11:26 am 218. Proofreading and Ribavirin-5′-Monophosphate Excision by SARS-CoV Polymerase/Exonuclease Complex.  
Lorenzo Subissi, Etienne Decroly, Isabelle Imbert, Bruno Canard  
AFMB UMR7257 CNRS and Aix-Marseille University, Marseille, PACA, France

Joanna J. Zmurko, Johan Neyts, Kai H. Dallmeier  
KU Leuven, Leuven, Flemish Brabant, Belgium

11:42 am 220. A Coxsackievirus Mutant Facilitates an Alternative Site for Replication Under PI4KIIIβ Inhibition.  
Charlotte Melia¹, Hilde M. van der Schaar², Ronald W. A. L. Limpens¹, Eric J. Snijder¹, Abraham J. Koster¹, Frank J. M. van Kuppeveld², Montserrat Bárcaña³  
¹Leiden University Medical Center, Leiden, South Holland, Netherlands, ²University of Utrecht, Utrecht, Utrecht, Netherlands

11:50 am 221. An Enterovirus 71 Mouse Model with Central Nervous System Involvement.  
Aloys Tijsma¹, David Franco¹, Rolf Hilgenfeld², Mathy Froeyen¹, Johan Neyts¹  
¹Rega Institute for Medical Research, Leuven, Belgium, ²University of Lübeck, Lübeck, Germany

Pietro Scaturro¹, Iuni Trist², David Paul¹, Chelsea M. Byrd³, Robert Jordan³, Andrea Brancale², Ralf Bartenschlager¹⁴  
¹Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany, ²School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, ³SIGA Technologies, Inc., Corvallis, Oregon, United States, ⁴German Center for Infection Research, Heidelberg, Germany
NEW DRUG SCREENING TECHNOLOGIES AND EMERGING INFECTIONS

Chair(s): Jinhong Chang, M.D. Ph.D. and Mike Bray, M.D.
2:30 pm – 3:15 pm
BALLROOM A

Organotypic Epithelial Raft Cultures as Versatile and Faithful Environments for Antiviral Drug Testing
Thomas R. Broker, Jei-Hwa Yu, Hsu-Kun Wang, Nilam Sanjib Banerjee, Louise T. Chow
University of Alabama at Birmingham, Birmingham, USA

Our objectives have been to establish the complete infection cycle of human papillomavirus (HPV) and to determine potential targets for anti-viral drug discovery. Three-dimensional organotypic ‘raft’ cultures can be produced from epithelial tissue explants, from HPV lesions or from isolated primary human keratinocytes (PHKs) placed on a dermal equivalent consisting of a collagen matrix with embedded fibroblasts. When grown at the liquid medium-air interface, keratinocytes stratify and differentiate to recapitulate the tissue of origin. Full-length HPV-18 genomes can be introduced into PHKs using Cre/loxP-mediated site-specific excision from transfected plasmids. Such infected cells undergo the full profile of HPV gene expression, vegetative DNA replication and production of abundant mature virions. These can productively infect fresh keratinocytes developed into 3D epithelia. The experimental model system is being used to investigate virus-host cell interactions and to validate low molecular weight inhibitors of viral DNA amplification. As one example, HPV DNA replication is regulated in a unique fashion, with tight control over the cytoplasmic–nuclear shuttling of the viral DNA helicase E1 that is exerted by phosphorylation of several serine residues. Inhibitors of the responsible host kinases block nuclear entry or retention of E1, effectively preventing HPV-18 amplification. Other classes of inhibitors have been validated as part of the NIAID Collaborative Antiviral Testing Group. Comparable raft cultures produced from HPV-immortalized or transformed cancer cell lines establish neoplastic epithelia and are being used to investigate potential anti-HPV tumor agents on the basis of our understanding of viral oncogene functions. Conclusions: Using 3D organotypic cultures, we are systematically finding effective inhibitors of HPV vegetative replication and virion production as well as agents that block tumor cell growth or survival. Globally, there is a major unmet need for effective and affordable topical agents to treat benign and neoplastic HPV lesions.

Identification of Human Neutralizing Antibodies against MERS-CoV and Their Role in Virus Adaptive Evolution
Sudhakar Agnihotram1, Xian-Chun Tang2, Yongjun Jiao2, Jeremy Stanhope2, Rachel Graham1, Eric Peterson2, Ralph Baric1, Wayne Marasco2
1Departments of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, USA, 2Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, USA

The newly emerging Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causes a severe acute respiratory syndrome (SARS)-like disease with ~43% mortality. Zoonotic transfer of MERS-CoV to humans is suspected given the recent detection of virus in dromedary camels. In addition, little is known about the role of human neutralizing antibody (nAb) pressure as a driving force of MERS-CoV adaptive evolution. Here we used an ultra-large non-immune human antibody-phage library and novel
panning strategy to identify seven human nAbs against the receptor binding domain (RBD) of MERS-CoV spike protein. These nAbs bind to three different epitopes in RBD-hDPP4 interface with subnano/nanomolar binding affinities and block the binding of MERS-CoV spike with its hDPP4 receptor. Escape mutant assays identified 5 amino acid residues that are critical for neutralization escape. Despite the close proximity of the three epitopes on the RBD interface, escape from one epitope did not have a major impact on neutralization by antibodies (Abs) directed to a different epitope. Importantly, the majority of escape mutations had a negative impact on hDPP4 receptor binding and viral fitness. These results provide the first report so far on human nAbs against MERS-CoV that may contribute to MERS-CoV clearance and evolution. Moreover, in the absence of a licensed vaccine or antiviral for MERS, this panel of nAbs offers the possibility of developing human monoclonal Ab (mAb) based-immunotherapy.

18 Advances in the development of T-705 for the Treatment of Arenaviral Hemorrhagic Fevers
Brian B. Gowen¹, Jonna B. Westover¹, Eric J. Sefing¹, Kevin W. Bailey¹, David Safronetz², Yousuke Furuta³, Donald F. Smee¹
¹Utah State University, Logan, USA, ²Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, USA, ³Toyama Chemical Co., Ltd., Tokyo, Japan

Arenaviruses are responsible for severe hemorrhagic fever (HF) syndromes with high morbidity and mortality. Five New World arenavirus species are known to cause fatal disease in the Americas, including Argentine, Bolivian, and Venezuelan HFs. The Lassa fever (LF) arenavirus is endemic in western Africa and has been imported to the United States and Europe by exposed travelers. Ribavirin has been shown to provide some protection in cases of LF, but remains largely unproven with only limited data for other arenaviral HFs. Without a specific treatment in sight, the HF arenaviruses pose a significant threat to public health. We have shown that T-705 inhibits arenavirus replication in cell culture and can protect against acute arenaviral diseases in rodent models. The present studies build on these promising results by expanding research into combination therapies and studies with LF virus. Here, we show that combined suboptimal doses of T-705 and ribavirin act synergistically to reduce mortality associated with Pichinde arenavirus infection, a model of LF. We also demonstrate for the first time T-705 antiviral activity against authentic LF virus in cell culture with inhibitory concentrations in the µM range. New data from studies evaluating T-705, alone or in combination with ribavirin, in models of Argentine HF and LF will be discussed. ACKNOWLEDGEMENT: Supported by NIH contract HHSN272201000039I, the Rocky Mountain RCE grant U54 AI-065357, and the Division of Intramural Research, NIAID.

19 Evaluation of Luciferase and GFP-expressing Nipah Viruses for Rapid Quantitative Antiviral Screening
Michael K. Lo, Stuart T. Nichol, Christina F. Spiropoulou
Centers for Disease Control and Prevention, Atlanta, USA

Nipah virus (NiV) outbreaks have occurred in Malaysia, India, and Bangladesh, and the virus continues to cause annual outbreaks of fatal human encephalitis in Bangladesh due to spillover from its bat host reservoir. Due to its high pathogenicity, its potential use for bio/agro-terrorism, and to the current lack of approved therapeutics, NiV is designated as an overlap select agent requiring biosafety level-4 containment. Although the development of therapeutic monoclonal antibodies and soluble protein subunit vaccines have shown great promise, the paucity of effective antiviral drugs against NiV merits further exploration of compound libraries using rapid quantitative antiviral assays. As a proof-of-concept study, we evaluated the use of fluorescent and luminescent reporter NiVs for antiviral screening. We constructed and rescued NiVs expressing either Renilla luciferase or green fluorescent protein, and characterized their reporter signal kinetics in different cell types as well as in the presence of several inhibitors. The 50 percent effective concentrations (EC50s) derived for inhibitors against both reporter viruses are within range of EC50s derived from virus yield-based dose-response assays against wild-type NiV (within 1 Log10), thus demonstrating that both reporter NiVs can serve as robust antiviral screening tools. Utilizing these live NiV-based reporter assays requires modest instrumentation, and circumvents the time and labor-intensive steps associated with cytopathic effect or viral antigen-based assays. These reporter NiVs will not only facilitate antiviral screening, but also the study of host cell components that influence the virus life cycle.
20  
Antiviral Optimization of a HCV NS5A Inhibitor ZN6168 with Picomolar Pan-Genotypic Activity and Excellent Safety
Zheng-Yun James Zhan1,2, Qing Li2, Guoyan Zhang2, Hua Yan1
1AB Pharma Ltd., Shanghai, China, 2Zannan SciTech Co., Ltd., Shanghai, China

BACKGROUND: NS5A of hepatitis C virus (HCV) is a non-structural protein that is considered essential for viral replication and infectivity. It has been intensively studied for globally urgent need of new effective HCV inhibitors since 2000, and we have developed several kinds of novel antiviral compounds highly potent and safe as an NS5A inhibitor.

RESULTS: This presentation discloses a novel optimized antiviral compound ZN6168 as one of the most competitive HCV NS5A inhibitors developed by AB-Zannan team. It was found that the selected HCV inhibitor ZN6168 had not only high potency (EC50: picomolar potency, 1-50pM for GT-1a, GT-1b, GT-2a, GT-3a and GT-4a, respectively) but also excellent PK and safety in rats. There was no test-article related side effect determined in combination of ZN6168 with different kinds of potential targets such as hERG, Cytochrome P450, etc., respectively. Its metabolic stability in human liver and plasma is very good (T1/2: >120min), and it could be formulated as once-daily dosing tablet. Regarding the safety issue, there was no any death, no any serious drug-related toxicity and adverse events observed during safety study in rats with oral dosing levels 50-2000mg/kg/day, respectively. Moreover, there were no other adverse events observed in the blood examination, clinical biochemistry, organ coefficient, electrocardiogram (ECG), pathological and tissue microscopic examinations in rats and/or monkeys, respectively. CONCLUSIONS: All antiviral toxicity and PK results indicated that ZN6168 had picomolar potency, excellent safety and PK, so ZN6168 appeared better than another reported NS5A inhibitor BMS790052. Moreover, ZN6168 is ongoing for more preclinical studies, and our goal is to develop a leading anti-HCV combination therapy with our optimized pan-genotypic NS5A inhibitor ZN6168 and another leading NS3 inhibitor ZN2007.

21  
Activation of Intracellular Viral Sensors by the Anti-Hepatitis Agent SB 9200 – Implications for Broad-Spectrum Antiviral Activity
R.P. Iyer1, A. Sheri1, R.K. Pandey1, S. Padmanabhan1, B.E. Korba2, S. Bose3, M.E. Cunningham4, G.R. Foster4
1Spring Bank Pharmaceuticals, Milford, USA, 2Georgetown University Medical Center, Division of Molecular Virology and Immunology, Washington, USA, 3University of Texas Health Sciences Center, San Antonio, USA, 4The Liver Unit, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

INTRODUCTION: Acute and chronic infections caused by RNA and DNA viruses constitute a major worldwide public health crisis affecting millions of people. Only a limited arsenal of antiviral agents exists and the problem is further compounded by rapid emergence of resistance to antiviral drugs and dose-limiting toxicities. Therefore, new anti-viral drugs with novel mechanisms of action are urgently needed. SB 9200 is a nucleotide compound with potent anti-HBV activity against wild-type and resistant HBV mutants. SB 9200 has a novel mechanism of action involving the activation of RIG-I and NOD2, the cytosolic sensors of RNA viruses. To explore its antiviral spectrum, the activity of SB 9200 against the RNA viruses, HCV, RSV and Norovirus, were evaluated.

METHODS: Anti-HCV activity of SB 9200 was assessed in the “Capture fusion assay” using THP-1 cells infected with serum from chronically infected patients with HCV genotypes 1-6. Five days following treatment with SB 9200, HCV RNA was quantitated using PCR. Anti-RSV activity was assessed using human lung epithelial A549 cells infected with RSV. At 16 h, following treatment with SB 9200, RSV titer was
assessed by plaque assay using CV-1 cell monolayers. Activity of SB 9200 against Norovirus was assessed using the HG23 cell line infected with NoV. Assays involving RNA hybridization and quantitative PCR were used to evaluate antiviral activity.

RESULTS: SB 9200 showed potent pan genotypic antiviral activity against HCV (EC\(_{50}\) 25 to 180 nM), RSV (EC\(_{50}\), 250 nM), and Norovirus (EC\(_{50}\), 2 mM). The broad spectrum anti-viral activity of SB 9200 is consistent with its unique mechanism involving the selective activation of RIG-I and NOD2 in virus-infected cells resulting in enhanced intracellular production of IFN and induction of antiviral state in cells. SB 9200 is currently in human clinical trials against HCV.

22 RSV Envelope Pseudotyping and Cell-Cell Fusion Assays Enable Rapid Phenotypic Analysis of RSV Fusion Protein Resistance Mutations
Kirsten Stray, Shreya Pramanick, Krista McCutcheon, Robert Jordan, Tomas Cihlar, Michel Perron
Gilead Sciences, Inc., Foster City, USA

Respiratory syncytial virus (RSV) causes acute respiratory tract infections and is a common cause of pediatric hospitalizations. The RSV fusion (F) protein is essential for virus replication since it mediates the fusion of viral and cellular membranes during virus entry, making it an attractive target for neutralizing antibodies and small molecule inhibitors. While specific mutations in the F protein have been identified that confer resistance of RSV to either monoclonal antibodies or small molecule inhibitors, effective tools for the phenotypic characterization of these mutations are lacking. Currently, only a lengthy and cumbersome reverse-engineered RSV recombinant system is available for these phenotypic analyses.

Here we describe the development and validation of two novel RSV phenotypic assays, an RSV F-pseudotyped HIV reporter assay and an RSV F-dependent cell-cell fusion assay, designed to streamline the phenotypic characterization of RSV F mutations. High levels of F protein expression and its efficient incorporation into pseudovirions as well as cell membranes were essential to maximize the signal-to-noise ratio and reproducibility of the phenotypic assays. This was achieved by codon optimization of the F gene combined with a unique truncation of the F cytoplasmic tail. The neutralizing antibody, palivizumab, and a small molecule RSV fusion inhibitor, VP-14637, exhibited consistent activity in the F pseudovirus assay (EC\(_{50}\) = 0.01 µg/mL and 0.2 nM, resp.), the F-dependent cell-cell fusion assay (EC\(_{50}\) = 0.04 µg/mL and 5.5 nM, resp.), and the infectious virus replication assay (EC\(_{50}\) = 0.05 µg/mL and 1.6 nM, respectively). F protein mutations conferring the antiviral resistance of infectious recombinant RSV to palivizumab (N262Y, N268I, K272M, and S275F) or VP-14637 (K399I, T400A, D486N, E487D, and F488Y) were also associated with substantially reduced activity of the tested inhibitors both in the pseudovirus assay and in the cell-cell fusion assay. These data support the use of the two streamlined systems for the effective phenotypic characterization of novel RSV F mutations.

23 Expressed Drug-Resistant HIV Subpopulations Identified by Surface Marker Immunocapture
S Malik\(^1\), L Morris\(^2\), C Yang\(^3\), C Zeh\(^1\), J Kiarie\(^4\), J Stringer\(^5,6\), P Weidle\(^1\), J Johnson\(^1\)

\(^1\)Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, USA, \(^2\)National Institute for Communicable Diseases, Johannesburg, South Africa, \(^3\)Division of Global HIV/AIDS, Centers for Disease Control and Prevention, Atlanta, USA, \(^4\)Kenyatta National Hospital, Nairobi, Kenya, \(^5\)CIDR, Lusaka, Zambia, \(^6\)U North Carolina, Chapel Hill, USA

Drug-resistant HIV can decrease the effectiveness of antiretroviral therapy (ART) and pose a risk for transmitted resistance. Host membrane proteins incorporated in the envelope of HIV virions can reveal cellular origin. We developed a novel immunomagnetic capture assay to identify cellular sources of drug-resistant virions. Plasma from pre- and post-single-dose nevirapine (NVP) exposed HIV-infected women (n=10 pairs) were applied to a sequential capture assay using 11 antibodies targeting host membrane proteins to differentiate HIV derived from different cell types. Nine of the 10 women initiated ART with 8 experiencing virologic failure. HIV RT sequences at virologic failure were compared to capture genotypes. Genotypes from follicular dendritic cells or splenic precursors and effector memory cells exhibited greater divergence within
individuals while genotypes from homing Mc, DC and activated T cell sequences had greater nucleotide ambiguity. Although K103N was found in sequences at virological failure, it was not observed in all compartments prior to treatment for all individuals. Additionally, at virologic failure, minority-level mutations not represented in the plasma bulk sequences, such as Y188C and G190A, were also found originating from specific cell types. Genotypes of NVP-exposed women at virologic failure showed the greatest similarity to HIV sequences with resistance originating from classical Mc/macrophages, homing Mc, DC and/or activated T cells. We developed an HIV immunomagnetic capture algorithm capable of isolating HIV virions originating from different immune cell types. We found that distinct drug-resistant variants can be expressed from diverse cellular reservoirs, with most expressed mutations undetected by bulk sequencing originating from classical Mc/macrophages and homing Mc. Further examination of these subpopulations may improve our understanding of resistant variant persistence, transmission, and responses to ART.

24 The Mode of Action of ST-246 Primarily Involves F13L in Orthopoxviruses and Also B5R in Camelpox Virus
Sophie Duraffour1, Maria M. Lorenzo2, Gudrun Zöller3, Dimitri Topalis1, Dennis E. Hruby4, Rafael Blasco2, Hermann Meyer3, Graciela Andrei1
1Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium, 2Departamento de Biotecnologia, INIA, Madrid, Spain, 3Bundeswehr Institute of Microbiology, Munich, Germany, 4SIGA Inc, Corvallis, USA

ST-246 is one of the key antivirals for the therapy of orthopoxvirus (OPV) infections. While investigating its mode of action, we identified amino acid changes in the F13L of 2 drug-resistant vaccinia virus clones (VACV, G277C or I372N), 2 cowpox virus clones (CPXV, D283Y or H194N+SVK303-305) and 2 camelpox virus clones (CMLV, F25V+I372N or G277C+I372N). These changes were responsible for drug-resistance as evidenced by producing recombinant viruses. Interestingly, one ST-246-resistant CMLV strain had no mutation in the F13L gene. Examination of other genes involved in virus wrapping (A27L, A33R, A34R and B5R) revealed a frameshift mutation due to the insertion of 5 nucleotides in the cytoplasmic tail of B5R. The role of B5R in ST-246 inhibitory activity appeared restricted to CMLV, as proven through the use of various B5R-mutated recombinant VACVs and CMLVs. We showed that the presence of the B5R-mutation altered the distribution of the B5R protein and not that of the F13L protein in CMLV, and that ST-246 treatment did not further delocalize mutant B5R while it did with wild-type B5R. These results suggest that the interaction between F13L and B5R is involved in ST-246 resistance. A putative tridimensional model of the interaction of ST-246 with F13L revealed that the drug may interfere with the phospholipase motif (HKD) of F13L which is needed for protein localization and wrapping. We finally showed that none of the identified mutations were naturally occurring polymorphisms in the F13L of 65 newly sequenced CPXV clinical isolates and 99 NCBI-OPVs. In conclusion, we demonstrated that OPV inhibition by ST-246 occurred through direct interaction with F13L and highlighted the role of an additional partner in CMLV, B5R. Besides providing data that will be helpful in clinical settings, our findings revealed fundamental features in the wrapping process of OPVs.
POSTER SESSION I
4:30 pm – 6:00 pm
BALLROOM C

25 Emergence of Genetically Variant Hepatitis C Virus Population in Patients with Low Viral Loads (<250 IU/mL), Pakistan
Muhammad Ali, Muhammad Idrees
University of The Punjab, Lahore, Pakistan

Mutations in NS5B gene of Hepatitis C virus (HCV) have been reported in patients undergoing antiviral therapy. In the present study, we report emerging clade of HCV-3a in patients administered with IFN plus ribavirin therapy for 24 weeks and having low viral loads (< 250 IU/mL).

Mutations D/N244E, K304R, N/K307G, Q/T329V and A338V were found associated with these emerging strains. This distinct HCV could be associated with the increased antiviral drug pressure.

26 Discovery and in vivo evaluation of PI4KIIIb Inhibitors for Picornavirus Infections
Martin Andrews¹, Dale Mitchell², Herve van der Poel², Hilde van der Schaar⁴, Frank Van Kuppeveld⁴, Pieter Leyssen³, Johan Neyts³, Armando da Palma³
¹Galapagos NV, Mechelen, Belgium, ²BioFocus plc, Chesterford Research park, United Kingdom, ³Rega Institute, Leuven, Belgium, ⁴Nijmegen University, Nijmegen, Netherlands

Human rhinovirus (HRV) is one of the major causes of exacerbation of inflammation in lung diseases such as COPD and asthma, which can result in high cost hospital admissions for treatment. Avoiding this problem would benefit both patients and health care systems generally. We have previously reported the discovery of a series of compounds targeted against PI4KIIIb, a confirmed host factor in picornavirus family replication. We had demonstrated that a compound from the series reduced the severity of Coxsackie virus induced pancreatitis in a mouse model. We now demonstrate further the scope and limitations of an picornavirus inhibitor that targets this enzyme. Drug resistant variants were selected by continuous culture in the presence of compound, and were found to carry the mutation H57Y in the 3A protein, the same mutation selected for by enviroxime. The 3A protein has been reported to interact with PI4KIIIb, and we observed inhibition of this kinase by the compounds. The series was further optimised focusing particularly on the ADME and PK profiles. A small number of compounds were selected for advanced assessment, including testing in the prophylactic mouse pancreatitis model. The in vitro ADME-tox profile of the series appeared good, and a lead compound was selected for advanced PK testing which showed good exposure in multiple species. Literature reports during LO indicated potential impact of the MoA on B cell biology. Both in vitro and in mice, effects were observed that were in line with such mechanism. To establish a possible therapeutic margin, the compound was next evaluated in a lethal Coxsackie virus model in SCID mice. Surprisingly, and in contrast to the effect previously observed, the compound resulted in a very limited protective antiviral effect in this model. In conclusion, PI4KIIIb does not appear to be a suitable target for the development of anti-picornaviral drugs.
27 Glycine Ester Derivatives of 2H-pyran-3-carboxylic Acid as Possible Anti-HSV Agents
Chandralata Bal¹, Srinivas Karampuri¹, Durbadal Ojha², Paromita Bag², Debprasad Chattopadhyay², Ashoke Sharon¹
¹Department of Applied Chemistry, Ranchi, India, ²ICMR Virus Unit, ID & BG Hospital, Kolkata, India

The clinical management of herpes virus diseases is limited due to ineffective clearance of virus particles and frequent emergence of drug-resistant viruses, particularly in immune-compromised patients, pregnant women and neonates. In our continued quest for new anti-HSV lead, glycine ester derivatives of 2H-pyran-3-carboxylic acid were synthesized and evaluated for anti-HSV activities on African green monkey kidney cell (Vero cells, ATCC, Manassas, VA, USA). Among several synthesized compounds, SK-I [EC₅₀ = 6.5 µg/ml (HSV-1) and 8.8 µg/ml (HSV-2)] and SK-II [EC₅₀ = 16.4 µg/ml (HSV-1) and 18.5 µg/ml (HSV-2)] have shown considerable activity with their CC₅₀s 74.8 µg/ml and 166.2 µg/ml respectively. 100% inhibitory concentrations were determined from dose-dependent activity on HSV post-infection. Investigation of virucidal effects, inhibition of viral penetration through plaque assay showed that they both were unable to inactivate (at their 100% inhibitory concentration) and inhibit viral penetration. Time course analysis showed that SK-I and SK-II inhibit HSV-1 and HSV-2 within 2-6 h post infection, i.e., during the early period of virus multiplication at EC₅₀ dose. Whereas no inhibition in pre-infection or co-infection was observed.

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28 Furthering Our Understanding HIV-1 Tropism in Late Stage Disease
Maria M Bednar¹,², L Ping¹,², SB Joseph¹,², LP Kincer¹,², MS Cohen¹,³, R Swanstrom¹,²,⁴
¹UNC Center of AIDS Research, Chapel Hill, USA, ²Lineberger Comprehensive Cancer Center, Chapel Hill, USA, ³Division of Infectious Diseases, School of Medicine, Chapel Hill, USA, ⁴Department of Biochemistry and Biophysics, Chapel Hill, USA

Understanding of HIV-1 tropism and evolution is paramount to understanding pathogenesis, latency, and disease progression. Entry phenotypes of HIV-1 isolates are currently being redefined though the use of a new assay, which defines entry phenotype of HIV-1 by the ability to use the primary surface receptor, CD4. Viral Env protein ability to utilize low densities of CD4 is a necessary step to become macrophage-tropic. We have identified examples of M-tropic virus infrequently in the CSF and genital tract. However the extent of M-tropic virus abundance in other compartments, such as the blood, remains unknown. We set out to determine if M-tropic viruses ever reach a point of systemic infection. Viral RNA was isolated from blood plasma samples from viremic subjects infected with either subtype B or subtype C HIV-1, and who were late in disease progression with CD4+ T cell counts of < 100 cell/mm³. To date, no examples of HIV-1 Env protein derived from virus in the blood has been found capable of using low levels of CD4, and therefore no M-tropic virus have been found in the blood. However, an emerging intermediate level of CD4 usage may indicate that these viruses have started to progress down a pathway to M-tropic virus evolution. We validated that we are examining late stage subjects with the identification X4 lineages in over 50% of the subjects. We are continuing to expand the sample size of this analysis. Macrophage-tropic viruses were previously considered to be major contributors to infection and disease progression. Recent availability of a more robust assay allows identification of viruses that have undergone the evolutionary step to utilize low levels of CD4 and allowed us to identify M-tropic viruses as being rare and potentially limited in their evolution to specific compartments of the body. These results argue that there is unlikely to be a significant myeloid component to the latent reservoir, at least comprised of viruses that have evolved to enter cells using low levels of CD4 and as interpreted using virus found in the blood.
29 Decreased Ring Size in Cyclotriazadisulfonamide (CADA) Analogs with Preserved CD4 Down-Modulating and anti-HIV Activity

Thomas W. Bell¹, Emily D. Scarbrough¹, Victor Van Puyenbroeck², Dominique Schols², Kurt Vermeire²
¹Department of Chemistry, University of Nevada, Reno, USA, ²K U Leuven Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium

The small-molecule cyclotriazadisulfonamide (CADA) prevents HIV entry into target cells by down-modulating their surface CD4 receptor expression. More specifically, CADA inhibits co-translational translocation of nascent human CD4 across the endoplasmic reticulum (ER) membrane and subsequent biogenesis of CD4. The goal of the studies described here is to determine if the size of the ring backbone is a contributing factor to potency for CD4 down-modulation and if size variation could improve drug potency. Therefore, the 12-membered ring of the CADA lead compound is reduced to an 11-membered ring in the new analogs. Additional methoxy substituents on the sulfonyl side arms on the 11-ring backbone were also evaluated. Because QJ028, a CADA analog with a cyclohexylmethyl tail group, has increased potency for CD4 down-modulation, different 11-membered analogs with a cyclohexylmethyl tail were synthesized and tested. Evaluation of CD4 down-modulation performed in CHO cells transfected with a fluorescent CD4 fusion protein revealed enhanced activity for ES-US2 (IC₅₀ = 330 nM), an 11-membered analog with combined cyclohexylmethyl tail and methoxybenzenesulfonyl side arm. In addition, CD4 down-modulation of all 11-membered analogs in the T-lymphoid cell line MT-4 correlated well with anti-HIV-1 potency. Also, cytotoxities of all the new analogs were found to be negligible (CC₅₀ > 75 µM). These data demonstrate that ring size variation of CADA is feasible with preservation of CD4 receptor down-modulating activity.

30 Anti-enteroviral activity of Prozac, Amantadine, and Ribavirin

Kimberley S. Benschop¹, Marieke Hoogerwerf¹, Harrie van der Avoort¹, Erwin Duizer¹, Marion P. Koopmans¹,2
¹National Institute for Public Health and the Environment, Bilthoven, Netherlands, ²Erasmus Medical Center, Rotterdam, Netherlands

Recent reports have suggested that several drugs that are used to treat chronic conditions are able to inhibit replication of enteroviruses (EVs). These drugs include fluoxetine (Prozac ©) that is used to treat depression, amantadine used to treat Parkinson’s diseases, and ribavirin used to treat chronic hepatitis C virus infections. While EV-associated illness is primarily seen in children, virus circulation without clinical symptoms is quite common in older age groups. Therefore, patients undergoing this type of treatment may have undiagnosed EV infection, potentially leading to selection and circulation of resistant. We therefore analyzed the activity of fluoxetine and amantadine against endemic EV’s in the Netherlands. In the current study we tested the susceptibility of a panel of 5 EV species B strains (Coxsackie virus (CV)A9, CVB3, CVB4, Echovirus 9 and 11) and poliovirus (PV) 1 Sabin strain, all chosen for their clinical relevance. We set out to determine the EC₅₀ of fluoxetine and amantadine on a range of cell lines as EC₅₀ concentrations can vary between cell lines. Initial testing showed evidence of inhibition but in a strain dependent manner on HT-29 cells. Fluoxetine inhibited replication of all 5 EV-B strains tested at a mean EC₅₀ drug concentration of 19.22 µM (range 14.76- 32.2 µM), but showed no activity against PV replication (at >25 µM). Replication of the reference strains was not inhibited by amantadine (>250 µM). The fluoxetine EC₅₀ concentrations were found to be higher than previously shown by others on other cell lines. These cell-based differences may have an effect on resistance emergence in vitro as well as in vivo. Currently, we are investigating susceptibility of ribavirin and effect of cell type, as well as possible mechanisms of inhibition. Results of this ongoing work will be presented.
31  
**Cytotoxicity and Antiviral Activity of a New Fluorine-containing Derivatives of 1,2,3-triazoles**

Liubov Biliavska1, Svitlana Zagorodny1, Olga Povnitsa1, Yuriy Shermolovich2, Ganna Gudz2, Nadiya Nesterova1

1Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukrenia, 2Institute of Organic Chemistry NAS of Ukraine, Kyiv, Ukrenia

The fluorine-containing derivatives are heterocyclic compounds that contain 1,2,3-triazole as a nucleobase with various substituents: residues of trifluoromethyl (G6, G8, G10, G12), perfluoropropyl (G9, G13), tolylsulfonyl (all compounds); and a glycoside fragment is represented by fragments of 3-chloro-tetrahydrofuran (G8, G9), 3-chloro-tetrahydropyran (G6, G14), dihydropyran (G10) and dihydrofuran (G12, G13), ethoxyethyl (G15), 2-chloro-2-deoxy- D-arabinofuranose (G16), 2-chloro-2-deoxy- D-arabinopyranose (G17), -D-ribofuranose (G18, G19), tetrahydrofuran (G20, G21), 2-chloro-1-ethoxyethyl (G22), 2-chloro-1(2)-(2,2,2-trifluoroethoxy)ethyl (G23), (2,2,2-trifluoroethoxy)vinyl (G24), ethoxyethanol (G25). Cytotoxicity of investigated compounds was determined by using the MTT method in Hela, M.D.BK and Raji cells. It was showed that substance G16 was toxic for all mentioned cells; substances G6, G21 and G22 were the most toxic for MDBK and Raji cells. Low toxicity of substances G8, G9, G10, G11, G18, G19, G20, G25 were demonstrated for all cells (from 375 to1010 µg/ml). We have investigated the inhibitory effects of substances on type 5 human adenovirus (HAdV5) replication in vitro by reduction of the quantity of infected cells. The G8 and G10 compounds demonstrated a selective antiviral effect in Hep-2 cells, infected with HAdV5. Their EC50 being 16µg/ml and 70 µg/ml, respectively. Level of reproduction Epstein-Barr virus in the test cells was studied by using the polymerase chain reaction. Among the studied compounds only G9 have expressed anti-EBV effect (EC50 was 30 µg/ml). We have investigated the effects of substances on herpes simplex virus (HSV-1/US and HSV-2/BH) in MDBK cells by reduction of cytopathic effect of the virus, detectable by MTT method. Anti HSV-1/US activity was shown substance G8 and G9 (EC50 was 50 µg/ml and 7,5 µg/ml, respectively). Anti HSV-2/BH activity was only substance G9 (EC50 =25 µg/ml).

32  
**Development of a DuoGel for Vaginal and Rectal Delivery of Microbicide Products**

AD Boczar1, CA Buchholz1, L Yang1, S Nugent1, A Ham1, CS Dezzutti2, KW Buckheit1, RW Buckheit Jr.1

1ImQuest BioSciences Inc, Frederick, USA, 2Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, USA

It has been over 30 years since the first cases of HIV/AIDS and prevention of infection through vaccination remains elusive. Thus, alternative products, technologies and strategies have emerged, including pre-exposure prophylaxis through oral ARVs and vaginal and rectal delivery of topical microbicides to prevent sexual transmission of HIV. It is well understood that men and women engage in receptive anal intercourse and women engage in both vaginal and anal intercourse during the same sexual encounter, creating a strong rationale for the development of microbicide delivery strategies targeting both vaginal and rectal routes of transmission in order to reduce HIV transmission. ImQuest is actively developing the NNRTI IQP-0528 as a microbicide agent based on its high potency, multiple mechanisms of antiviral action, and its ability to act in an additive or synergistic manner with other microbicide products. We have developed a safe HEC-based vaginal gel and anticipate submitting an IND for clinical development of this vaginal gel in early 2014. We have continued the development of the microbicide IQP-0528 with a new focus on a product which safely delivers this single agent to both compartments (DuoGel) as well as a combination DuoGel comprised of IQP-0528 and tenofovir. Based on the IQP-0528 vaginal gel and a defined product profile for a DuoGel, a series of single–agent and combination DuoGels having the defined osmolality, pH and viscoelastic properties to be safely used in both the vagina and rectum have been produced. Utilizing well established in vitro and ex vivo microbicide development assays we assessed the efficacy and toxicity of each of the placebo and API-containing gels. The microbicidal activity of these gels was not affected by the presence of vaginal and seminal simulants. No toxicity of the gels was observed to representative cell lines, epivaginal tissue, the normal flora Lactobacillus, or cervical or colorectal explant tissue. Based on these biological data a single agent DuoGel is being advanced to an IND and human clinical testing and a combination IQP-0528/Tenofovir DuoGel is being developed.
The Influence of Electromagnetic Fields of the Extra-Low Frequency on the Infectious Activity of the Influenza Virus and Staphylococcus Aureus

B Bogomolny¹, V Barzinsky¹, T Grydina², A Fedchuk², L Mudryk², V Lozitsky²
¹Corporation Informational Medicine, Kyiv, Ukrenia, ²Mechnikov Ukrainian Anti-Plague Research Institute, Odesa, Ukrenia

The researches like that became possible with appearance of new class of devices allowing to registrate and reproduce weak electromagnetic fields (EMF) of extremely low power in very low frequency band. The hardware and software “SCS-BARS” is one of such devices. The registration of electromagnetic vibrations in “SCS-BARS” is carried out in low frequency band (less than 30kHz). The aim of this research was studying influence of own spectra of EMF of extremely low power in the very low frequency band on replication of influenza virus and on growth of S.aureus. Autospektal fields (ASF) of the objects were recorded and their impact on virus and bacterial agents carried out in vitro in the inverse mode (IM). With the “SCS-BARS” we influenced with the ASF of object, recorded right before influence on influenza virus A/Hong Kong/1/68(H3N2) in the IM during 30 min. The control is carried out similar to the test without treatment by “SCS-BARS”. The qualities of infective viruses in experiment and control practically did not differ after 8hrs at 37°. After 24hrs viral infective titers in experiment were less on 1.0 log10 TID50 than in control. The greatest distinctions in growth of S.aureus were registered in 24hrs after the exposure and by the point of 48hrs the difference in microbial number between the control and experiment decreased. The average indices of optical density of control samples of S.aureus ATCC 25923 and S.aureus 2781 in 24 and 48hrs were lower than the control at average by 0.57±0.10 and 0.75±0.13 optical density units (ODU) by the McFarland. It corresponds to 4.1±0.1 colony-forming units, ml (CFU/ml) and 4.7±0.1 CFU/ml. This difference was more pronounced at S.aureus ATCC 25923 in 48hrs at exposure during 30min. The difference between the control and experiment averaged 0.67±0.12 EOP or 5.1±0.1 CFU/ml. So a single exposure of ASF on studied influenza virus and S.aureus strains inhibits their growth/replication in vitro. The obtained results prove a necessity of conducting further researches both for studying mechanisms of EMF on causative agents, and for development of new methods for anti-infection therapy.

Use of the ICCA to Predict Dosing of HIV Microbicide Products Required for Virus Sterilization in Target Tissue

CA Buchholz¹, KW Buckheit¹, C Shetler², CS Dezzutti², PM Mesquita³, BC Herold³, RW Buckheit Jr.¹
¹ImQuest BioSciences, Frederick, USA, ²Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, USA, ³Albert Einstein College of Medicine, Bronx, USA

The historical dosing strategy employed for HIV prevention products has been to flood target tissue with high nontoxic concentrations of a product. Continuation of this strategy results from the lack of data from a successful clinical product or dose response studies in a predictive animal model with direct correlation to use in humans. We have hypothesized that sensitive in vitro assays can be used to define the required tissue concentration of a microbicide to totally prevent HIV transmission and/or spread of virus from initially infected sites. The microbicide transmission and sterilization assay (MTSA) has defined sterilizing concentrations of microbicide agents. For example 100 nM of the NNRTI IQP-0528 and 117 µM of the NtRTI tenofovir are required to sterilize a virus infected cell culture. Through continued evolution of the MTSA and efforts to better understand the biology of sterilization, we have developed an infectious cell center assay (ICCA) to more sensitively evaluate sterilization by microbicides under conditions in which these products must act. For IQP-0528 the ICCA yielded identical sterilizing concentrations as the MTSA (100 nM) and correlated the sterilizing concentrations with effective concentrations observed in antiviral and pharmacodynamic assays employing cervical explant tissue. Protection of mucosal tissue from HIV challenge required approximately 10 µM of formulated IQP-0528 (100 µM with unformulated IQP-0528). Performance of antiviral assays with lysed explant tissue that had been soaked with 10 µM IQP-0528 also yielded complete protection of target cells, indicating that the 10 µM dose delivered a sterilizing concentration of IQP-0528 to the tissue. A 1% (w/v) IQP-0528 gel would deliver 30 mM IQP-0528 to vaginal target tissue, representing 1,000-10,000-fold excess over the sterilizing concentration of IQP-0528. Our efforts now involve understanding the tissue pharmacokinetics of microbicides in order to better understand not only the concentration of product but the timing of dosing relative to the time of infection and rate of product uptake.
35 Development of Tandemers of the Antiviral Natural Product Griffithsin as HIV Prevention Agents
KW Buckheit¹, RW Buckheit Jr.¹, L Yang¹, AD Boczar¹, CA Buchholz¹, T Moulaei², A Alexandre³, BR O’Keefe³
¹ImQuest BioSciences Inc., Frederick, USA, ²Protein Structure Section, Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, USA, ³Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, USA

The antiviral agent Griffithsin (GRFT), derived from the red algae Griffithsia, is a 121-amino acid lectin which binds specifically to the HIV envelope glycoproteins, potently inhibiting the binding of HIV-1 to its target cells at picomolar concentrations. GRFT is a homodimer in its native state with each monomer possessing three carbohydrate binding sites. GRFT’s mode of action results in broad antiviral activity, including enveloped viruses such as SARS Coronavirus, HCV, and herpes viruses. GRFT is being developed as an anti-HIV topical microbicide in light of its potent activity, high thermostability, activity across a wide pH range, absence of toxicity and immunogenicity, and its amenability to large scale manufacturing. In order to further enhance the biological activity of GRFT, taking advantage of the known orientation and spacial organization of the monomeric GRFT units, we have constructed flexible tandemers of GRFT comprised of arrays of two (2MG, 2MG3), three (3MG) or four (4MG) GRFT monomers which are linked together with short amino acid chains. The anti-HIV activity of the tandemers is enhanced by up to 10-20 fold compared to native GRFT, most prominently with the 3MG or 4MG constructs. As a potential prevention agent, we demonstrate that the activity of these tandemers reproducibly extends to all geographically diverse HIV-1 subtypes (A through G and O), to viruses with defined co-receptor tropisms, and that the tandemers remain active in the presence of vaginal fluids and semen. GRFT and its tandemers also act in an additive to synergistic fashion with other potential HIV microbicide products. Mechanistically, tandemers exert antiviral activity through the cross-linking of HIV envelope spikes, as opposed to causing viral aggregation. Continued development of this new class of microbicides is highly warranted based on the need for new highly active and nontoxic prevention products with broad anti-infective potential.

36 Evaluation of PD 404,182 as an Anti-HIV and Anti-HSV Microbicide
Ana M. Chamoun-Emanuelli, Zhilei Chen
Texas A&M University, College Station, USA

PD 404,182 (PD) is a synthetic compound that was found to compromise HIV integrity via interaction with a non-envelope protein viral structural component (Chamoun, et al. 2012. Antimicrobial Agents Chemotherapy 56:672-681). The present study evaluates the potential of PD as an anti-HIV microbicide and establishes PD’s virucidal activity towards another pathogen – herpes simplex virus (HSV). We show that the anti-HIV-1 IC₅₀ of PD when diluted in seminal plasma is ~1 µM, similar to the IC₅₀ determined in cell culture growth media, and that PD retains full anti-HIV-1 activity after incubation in cervical fluid at 37°C for at least 24 hours. In addition, PD is non-toxic towards vaginal commensal Lactobacillus species (CC₅₀ > 300 µM), freshly activated human PBMC (CC₅₀ ~ 200 µM) and primary CD4⁺ T-cells, macrophages and dendritic cells (CC₅₀ > 300 µM). PD also exhibits high stability in pH-adjusted DPBS with little-to-no activity loss after 8 weeks at pH 4 and 42°C, indicating suitability for formulation for transportation and storage in developing countries. Finally, for the first time, we show that PD inactivates herpes simplex virus (HSV)-1 and -2 at submicromolar concentrations. Due to the prevalence of HSV infection, the ability of PD to inactivate HSV may provide an additional incentive for use as a microbicide. The ability of PD to inactivate both HIV-1 and HSV, combined with its low toxicity and high stability warrants additional studies for the evaluation of PD’s microbicidal candidacy in animals and humans.
37 Synthesis of Unsymmetrical Cyclotriazadisulfonamide (CADA) Analogs as Human CD4 Down-Modulating Antivirals
Reena Chawla¹, Victor Van Puyenbroeck², Dominique Schols², Kurt Vermeire², Thomas W. Bell¹
¹Department of Chemistry, University of Nevada, Reno, USA, ²KU Leuven Department of Microbiology and Immunology, Rega Institute for Medical Research, Leuven, Belgium

Cyclotriazadisulfonamide (CADA) compounds inhibit entry of HIV into host cells by down-modulating CD4 receptor expression. These compounds reduce cell surface CD4 expression levels by inhibiting human CD4 protein synthesis in a co-translational translocation-dependent way. Previously, structure-activity relationships (SAR) studies have shown that CADA compounds apparently interact with two different arenesulfonyl binding sites. In this study, we investigated the relationship between electron density of one of the sulfonamide side arms of CADA and CD4 down-modulation potency. A series of twenty six unsymmetrical CADA analogs having two different arenesulfonyl side-arms have been synthesized and evaluated for their CD4 down-modulation properties. The CADA analog CK147, having a cyclohexylmethyl tail group and one 4-N,N-dimethylaminobenzenesulfonamide side arm, was found to have highest potency towards CD4 down-modulation in CHO cells transfected with a fluorescent CD4 fusion protein (IC₅₀ = 60 nM) and in the T-lymphoid cell line MT-4 expressing human CD4 naturally (IC₅₀ = 140 nM). In addition, the CK147-induced reduction in CD4 correlated with enhanced anti-HIV-1 NL4.3 activity (IC₅₀ = 180 nM). Generally, evaluation of the analogs suggested that CADA compounds having a larger dipole moment in one side arm, and no hydrogen bond donor group, exhibit higher potencies for inhibition of CD4 receptor expression. We hypothesize that one of the binding sites in the biomolecular target of CADA also has a dipole moment that pairs with the side arm dipole of the compound.

38 Identification of FDA-approved Drugs That Inhibit Middle East Respiratory Syndrome Coronavirus Replication in Cell Culture
Adriaan de Wilde¹, Dirk Jochmans², Clara Posthuma¹, Jessika Zevenhoven-Dobbe¹, Martijn van Hemert¹, Bernadette van den Hoogen³, Johan Neyts², Eric Snijder¹
¹Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands, ²Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ³Viroscience, Erasmus Medical Center, Rotterdam, Netherlands

Coronaviruses infect a wide variety of human and animal hosts and are commonly associated with respiratory and enteric disease. The 2003 outbreak of severe acute respiratory syndrome (SARS) demonstrated for the first time the potentially lethal consequences of zoonotic coronavirus infection in humans. In 2012, a similar previously unknown coronavirus emerged and was named Middle East respiratory syndrome coronavirus (MERS-CoV). Thus far, its fatality rate (~40%) among 178 confirmed human cases is alarmingly high. Ten years after the 2003 SARS outbreak, registered drugs to treat coronavirus infections are still not available. Therefore, we set out to develop a cell culture-based assay to screen for antivirals against MERS-CoV, based on the pronounced cytopathology the virus causes in Huh7 and Vero cells. This assay in a 96-well format was initially used to confirm cyclosporin A and interferon-α as inhibitors of MERS-CoV replication in cell culture (De Wilde et al., J. Gen. Virol., 2013). We next screened a library of ~700 FDA-approved compounds for their antiviral activity against MERS-CoV. Six compounds were found to significantly inhibit MERS-CoV replication with EC₅₀ values in the low-micromolar range. These compounds were also found to inhibit the replication of two other coronaviruses, i.e. SARS-CoV and human CoV-229E, with comparable efficacy. Their mode of action is currently being investigated, after which the most potent compounds, alone or in combination with previously reported MERS-CoV inhibitors like interferon-α, will be subjected to further testing. Evaluation in a MERS small-animal model, once available, should be included to assess whether these compounds indeed broaden the therapeutic options to combat MERS or coronavirus infections in general. This research was funded by the European Union FP7 Program under SILVER grant agreement nr² 260644.
Investigating the Highly Potent D-carba-dT in Primer Extension Assays
Thiago Dinis de Oliveira¹, Anna Rath¹,², Andrea Rentmeister², Chris Meier¹
¹University of Hamburg, Hamburg, Germany, ²University of Münster, Münster, Germany

Carbocyclic nucleoside analogues are extremely attractive compounds in drug development due to their high biological activity against several viruses. The carbocyclic nucleoside D-carba-dT shows promising antiviral activity. In antiviral assays D-carba-dT reveals high activity against diverse viruses e.g. HIV-1, HIV-2 and VV. Here, we present biochemical assays of D-carba-dT using the human DNA polymerases β and γ. The human DNA polymerase β is involved in DNA repair and the human DNA polymerase γ is essential in the replication of mitochondrial genome.

Beside to gain insights in the mode of action of the human DNA polymerases in bypassing the “lesion” caused by the D-carba-dT, also possible point mutations were examined by performing primer extension assays.

In order to enable this study D-carba-dT has been synthesized on a stereoselective, chemo-enzymatic convergent approach according to a procedure developed by our laboratories.

Afterwards D-carba-dT was converted into the corresponding phosphoramidite, which could be used in oligonucleotide synthesis to prepare 30-mer oligonucleotides. To prove if these human DNA polymerases take D-carba-dT as a substrate, D-carba-dTTP was prepared using the cycloSal-method. In the following primer extension assays the incorporation of the nucleotides were investigated. The results indicate that the incorporation of D-carba-dT hampers the elongation of the growing DNA strand. Furthermore, the position of the D-carba-dT in the DNA strand reveals to be important for effectiveness of chain termination.

Prodrugs for the Delivery of Anti-Filoviral N-Alkyldeoxynojirimycin Derivatives
Yanming Du¹, Richard Lu¹, Fang Guo², Ju-Tao Guo², Timothy Block¹,², Bill kinney¹, Xiaodong Xu¹, Jinhong Chang²
¹Baruch S. Blumberg Institute, Doylestown, USA, ²Drexel University College of Medicine, Doylestown, USA

Filoviral hemorrhagic fevers are highly lethal diseases which can be caused by Ebola and Marburg viruses. Currently, there are no effective vaccines to be used for prevention and no effective antiviral interventions to manage the diseases. Therefore, a drug that is safe and effective against both viruses would be an enormous benefit. We have discovered a novel class of N-alkyldeoxynojirimycins (NADNJs), which demonstrated significant protection for the mice infected with Marburg virus and Ebola virus. However, our lead compounds showed short half life and low oral bioavailability in mice. In addition, GI distress is also another concern because it has been associated with imino sugar glucosidase inhibitors when used orally. Here we report our prodrug strategy to increase oral bioavailability and avoid off-target inhibition of intestinal glucosidases. Two types of prodrugs, alkyl esters for improving cell membrane penetration and amino acid esters for transporter delivery, were synthesized. Hydrolysis of these prodrugs to parent compounds under different enzymes were studied and complete and partial hydrolysis products were analyzed. ADME profiles of the lead compounds were evaluated. The efflux ratio of a parent compound, IHVR-17028, was dramatically improved through acetylation of the four hydroxy groups of the DNJ head piece in a Caco study.
41 | Discovery of Inhibitors of Middle East Respiratory Syndrome Coronavirus Infection

Julie Dyall¹, Chistopher C. Coleman², Brit J. Hart¹, Monique Laidlaw³, Lisa M. Johansen⁴, Peter B. Jahrling¹,², Lisa E. Hensley¹, Matthew B. Frieman²

¹IRF NIAID/NIH, Frederick, USA, ²University of Maryland School of Medicine, Baltimore, USA, ³EVPS NIAID/NIH, Frederick, USA, ⁴Zalicus Inc., Cambridge, USA

Middle Eastern respiratory syndrome-coronavirus (MERS-CoV) is an emerging virus, and to date no antiviral or therapeutic has been approved for treating patients. Since September 2012, 178 patients, including 74 deaths, have been attributed to infection with MERS-CoV. Currently, supportive care remains the only available treatment option. Outbreaks of emerging infections present the unique challenge of trying to select appropriate pharmacologic treatments in the clinic with little time available for drug testing and development. Repurposing of approved pharmaceutical drugs for new indications presents an attractive alternative for clinicians, researchers, and public health agencies. A library of 290 compounds was screened to identify drugs that inhibit MERS-CoV or the related human pathogen severe acute respiratory syndrome coronavirus (SARS-CoV). Selection of compounds for inclusion in the library was based on activity in other antiviral screens. The library presents a unique panel of drugs that are FDA approved, in clinical development, or have a well-defined cellular pathway as target. A total of 67 compounds showed activity against either MERS-CoV or SARS-CoV with no or low toxicity. Of these, 27 compounds showed activity against both viruses. The compounds belong to thirteen different classes of pharmaceuticals including inhibitors of estrogen receptors used for cancer treatment and dopamine receptor inhibitors used as antipsychotics. Six compounds with micromolar IC₅₀ values were chosen for characterization: gemcitabine hydrochloride, toremifene citrate, chlorpromazine hydrochloride, trifupromazine hydrochloride, imatinib mesylate, and dasitinib. The drugs identified in these screens provide new targets for in vivo studies as well as incorporation into ongoing clinical studies. Preliminary studies to characterize the mechanism-of-action in virological assays will be discussed.

42 | New Indolylarylsulfones as Potent and Broad Spectrum HIV-1 Non-nucleoside Reverse Transcriptase Inhibitors

Valeria Famiglini¹, Giuseppe La Regina¹, Antonio Coluccia¹, Andrea Brancale², José A. Esté³, Romano Silvestri¹

¹Sapienza University, Roma, Italy, ²Cardiff University, Cardiff, United Kingdom, ³Universitat Autonóma de Barcelona, Badalona, Spain

Acquired immune deficiency syndrome (AIDS) pandemic remain among the leading causes of death worldwide. HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key drugs of highly active antiretroviral therapy (HAART) in the clinical management of AIDS/HIV-1 infection. Our recent studies showed that indolylarylsulfones (IASs) bearing a cyclic moiety at the 2-carboxamide nitrogen linked through a short spacer group were endowed with potent antiretroviral activity (1). Thus, we have expanded the SAR studies by the introduction of a number of (hetero)aryl or heterocyclyl moieties at the 2-carboxamide nitrogen through a methylene/ethylene bridge (Figure 1) (2). Several new derivatives were highly active against HIV-1 replication in MT-4 cells with inhibitory concentrations in the low subnanomolar range. The most active compounds were highly effective against HIV-1 WT and mutant HIV-1 strains carrying resistance mutations to the commonly used NNRTI drugs nevirapine and efavirenz.

43 Synthesis of 2’-O,4’-C-Alkylene-Bridged Ribonucleosides and their Evaluation as Inhibitors of HCV NS5B Polymerase

Rebecca Glen¹, Benjamin A Mayes², Stephen Moore¹, Adel Moussa², Alistair Stewart²
¹Peakdale Molecular Ltd, Chapel-en-le-Frith, United Kingdom, ²Idenix Pharmaceuticals, Cambridge, USA

Oligonucleotides containing locked nucleic acid (LNA) nucleosides have found numerous therapeutic and biotechnology applications since their introduction by Imanishi¹ and Wengel.² To date, however, there have been no reports of the evaluation of the LNA nucleosides themselves in terms of activity against the HCV NS5B viral polymerase. The poster will describe the synthesis and biological evaluation of the previously unknown guanosine analogs bearing 2’-C-methyl and 5’-C-methyl modifications ¹ and ², along with the known LNA nucleosides ³-⁶ and ENA nucleoside ⁷. Key to the installation of the 2’-C-methyl and 5’-C-methyl (of ¹ and ²) was a one-pot oxidation-Grignard procedure to avoid formation of unreactive hydrates prior to alkylation. In addition, we developed an efficient partition coefficient chromatographic technique for separation of the 5’-C-methyl epimers of ¹³. With nucleosides ¹-⁷ in hand, we set about testing their biological activity against HCV. The free nucleosides were screened in a HCV cell-based replicon assay. The corresponding nucleoside triphosphates (TP) were screened against the purified HCV NS5B polymerase [1b wild type (WT) and 1b S282T mutant] in vitro. Of particular note was novel LNA-G-TP ¹, which exhibited potent inhibition with IC₅₀ values of 0.20 (1b WT) and 0.48 (1b S282T) µM. The full results of these studies will be presented in the poster.


44 Development of a High-Throughput Cell-based Antiviral Assay for Screening Inhibitors of Chikungunya Virus

Edwin Gong¹, Jean-François Bonfanti², Guenter Kraus¹
¹Janssen Infectious Diseases-Diagnostics BVBA, Johnson & Johnson Corporation, Beerse, Belgium, ²Janssen Research & Development, Val de Reuil Cedex, France

Chikungunya virus (CHIKV), a member of the Alphavirus genus of the Togaviridae family, is an enveloped positive-sense single-stranded RNA virus that is transmitted by Aedes mosquitoes. CHIKV re-merged around 2005-2006 and caused large-scale epidemics and millions of infection in Africa, the islands of the Indian Ocean, South and Southeast Asia, and northern Italy. The infection is still ongoing in many countries, especially countries in South and Southeast Asia. It causes painful arthritis-like symptoms that can last for months or even years. At present, there are no vaccines commercially available and no effective antiviral therapies are being developed for the treatment of CHIKV infection. In order to discover potential small-molecule inhibitors against CHIKV infection, we have developed a cell-based high-throughput antiviral assay for screening inhibitors against CHIKV. Our HTS assay utilizes a 384-well plate format and ATP/luminescence readout and can identify inhibitors at any stage of the virus replication cycle. The assay parameters were fully validated. We have screened more than 16000 compounds using this assay and ~100 hits were confirmed. These hits are currently being evaluated in the other profiling assays.
Identification and Characterization of Small Molecule Inhibitors of Dengue Virus Translation

Jason D Graci¹, Stephen P Jung¹, Guangming Chen¹, Chunling Wang², Mildred Galvez², Zhengxian Gu¹, Eva Harris², Joseph M Colacino¹
¹PTC Therapeutics, Inc., South Plainfield, USA, ²Division of Infectious Diseases, School of Public Health, University of California, Berkeley, Berkeley, USA

Dengue is the most common arthropod-borne viral infection of humans, with an estimated 96 million clinically apparent cases per year worldwide. Despite decades of research, a vaccine has yet to be approved and current treatment is only palliative in nature. Orally bioavailable direct acting antiviral agents that selectively inhibit dengue virus replication may therefore address an urgent global unmet medical need. We developed a high throughput screening assay based on our Gene Expression Modulation by Small molecules (GEMS™) technology to screen our chemical library and identify small molecules that can specifically inhibit dengue virus protein translation by acting through the untranslated regions (UTRs) of the genomic RNA. Using this technology, we have identified several early screening hits in structurally distinct chemical scaffolds that inhibit multiple dengue virus serotypes in infectious viral assays while exhibiting selectivity with respect to other viruses and the translation of human mRNA. Consistent with a mechanism of action mediated through the viral UTRs, results of initial studies demonstrated that these compounds inhibit an early stage in the viral replication cycle. These results point to the utility of the GEMS™ technology in the discovery of novel small molecule inhibitors of dengue and other RNA viruses.

Discovery of Novel HCV IRES Inhibitors by SHAPE-directed Screening

David Grawoig¹, Carly Sherrod², Fethullah Karabiber³, Oleg V. Favorov⁴, Dirk P. Dittmer², Kevin M. Weeks¹
¹Chemistry Department, UNC-Chapel Hill, Chapel Hill, USA, ²Microbiology and Immunology Department, UNC-Chapel Hill, Chapel Hill, USA, ³Department of Computer Engineering, Yildiz Technical University, Istanbul, Turkey, ⁴Biomedical Engineering, UNC-Chapel Hill, Chapel Hill, USA

Targeting RNA with small molecules is a promising approach for developing novel antiviral drugs. We developed an automated, high throughput selective 2’-hydroxyl acylation analyzed by primer extension (SHAPE) screen to identify small molecule binding to RNA at single nucleotide resolution. We applied this approach to the Hepatitis C Virus (HCV) internal ribosome entry site (IRES), a highly conserved RNA required for cap-independent viral translation. HCV IRES RNA is an attractive drug target that contains multiple, functionally critical, structured domains. Previous work has shown that 2-aminobenzimidazole derivatives bind HCV IRES domain IIA and inhibit viral translation. We performed a “proof-of-principle” screen of a focused library of 268 2-aminobenzimidazole-based compounds against full length HCV IRES RNA and identified >30 ligands that produced statistically significant changes in SHAPE reactivity. Importantly, several ligands inhibited IRES mediated translation in dual luciferase assays. Our results demonstrate that automated SHAPE screening is a powerful new method for identifying small molecule ligands for large, structurally complex RNA.
A Cell-based High Throughput Assay for the Discovery Of Compounds With Antiviral or Innate Immune Response-modulating Activity

Fang Guo1, Xuexen Zhao1,2, Yanming Du2, Andrea Cuconati2, Michael Goetz3, Timothy M. Block1,2,3, Ju-Tao Guo1, Jinhong Chang1

1Drexel University College of Medicine, Doylestown, USA, 2Baruch S. Blumberg Institute, Hepatitis B Foundation, Doylestown, USA, 3Natural Products Discovery Institute, Hepatitis B Foundation, Doylestown, USA

Virus infection of host cells is sensed by innate pattern recognition receptors (PRRs) and induces production of type I interferons (IFNs) and other inflammatory cytokines. These cytokines orchestrate the elimination of the viruses but are occasionally detrimental to the hosts. The outcomes and pathogenesis of viral infection are largely determined by the specific interaction between the viruses and their host cells. The aim of our study was to discover novel therapies against virus infection by screening and identifying compounds that either inhibit viral infection or modulate virus-induced cytokine response. The screening platform is a HEK293 cell-based reporter assay where the expression of a firefly luciferase gene is under the control of a human IFN- β promoter. We demonstrated that infection of the reporter cell line with a panel of RNA viruses activated the reporter gene expression that correlates quantitatively with the levels of virus replication and progeny virus production, and could be inhibited in a dose-dependent manner by either known antiviral compounds or inhibitors of PRR signal transduction pathways. Using dengue virus infection as a model, screening of a small molecule library consisting of 26,900 compounds identified both antiviral agents and inhibitors of PRR signal transduction. In addition, a pilot screening of a natural product library consisting of 2,000 crude extracts from plants, bacteria and fungi, followed by bioactivity-guided fractionation has identified nigericin as inhibitor of dengue virus infection. Our studies have thus proven the concept that the IFN- β promoter reporter assay can serve as a convenient high throughput screening platform for simultaneous discovery of antiviral and innate immune response modulating compounds against the infection of many different viruses.

Formulation Development of the DuoGel: a Dual Chamber Vaginal/Rectal Anti-HIV Microbicide Gel

Anthony Ham1, William Lustig1, Sean Nugent1, David Katz2, Charlene Dezzutti3, Ashlee Boczar1, Karen Buckheit1, Robert Buckheit1

1ImQuest BiSciences, Frederick, USA, 2Duke University, Durham, USA, 3Magee Womens Research Institute, Pittsburgh, USA

Background: The DuoGel, an anti-HIV gel microbicide formulation, is currently being developed as a single product for safe vaginal and rectal administration to address the high incidence of both vaginal and anal intercourse in the same sexual act by reducing the user complexity of maintaining separate dosage forms, as well as a product safely designed for the MSM population. Methodology: The DuoGels containing the NNRTI IQP-0528 were formulated from GRAS excipients approved for both vaginal and rectal administration and evaluated for physicochemical and biological properties. The pH and osmolality of the DuoGels were defined by a target product profile. DuoGel viscosity was measured under parallel plate geometry from 1E-5 s⁻¹ to 200 s⁻¹. In vitro drug release was performed in Franz cells through a cellulose membrane over 6 hours. The rheological spreading and distribution of 4 mL of DuoGel was evaluated for 2 hours under 1.143 lbf. In vitro toxicity of the DuoGels was performed against CaSki, HEC1A, and ME180 cell lines and Lactobacilli for 24 hours. In vitro efficacy was performed in PBMCs against HIV-1 infection for 7 days. The ex vivo toxicity, permeability, and efficacy of the DuoGels were performed in both polarized explant ectocervical and colorectal tissues. Results: The DuoGel formulation was developed and manufactured to a specific pH (6.00) and osmolality (300 mmol/kg) to accommodate rectal administration. The DuoGel formulation, with a viscosity of 85.57 ± 5.48 Pa·s at 1 s⁻¹, resulted in a gel distribution of 103.2 cm². The DuoGel produced an in vitro and ex vivo drug release rate of 80 ± 3 µg/cm² hr to prevent HIV-1 infection in both vaginal and rectal environments with an EC₅₀ value of 2.34 ± 0.49 ng/mL. The DuoGel formulation displayed no in vitro cellular or bacterial toxicity up to a high concentration of 1000 µg/mL. It also resulted in no loss of cellular tissue viability in both explant ectocervical and colorectal tissue. Conclusions: A gel formulation has been identified to have the potential to safely prevent HIV-1 infection in the vagina and rectum.
### 49 Development of Fullerene Poly-aminocaproic Acid for the Inhibition of Human Immunodeficiency Virus Type 1

**Tracy Hartman**, Lu Yang, Amanda Helfrick, Lev Rasnetsov, Robert Buckheit

1ImQuest Biosciences, Frederick, USA, 2CJSC Intelparm, Nizhny Novgorod, Russia

The development of highly active anti-HIV therapies has greatly suppressed virus replication and helped to prevent progression to AIDS in many infected patients. In the absence of a functional cure or effective vaccine, drug safety issues, and the ability of HIV to acquire resistance to drugs in current use, continued efforts to develop new anti-HIV compounds are warranted. Fullerenes, especially C60 derivatives, have unique carbon cage structures reported to bind the hydrophobic cavity of HIV protease, inhibiting the access of substrates to the catalytic site of the enzyme. Fullerene poly-aminocaproic acid (FPAA) was evaluated against a panel of 24 HIV-1 clinical isolates in human PBMCs and yielded EC50 values ranging from 1.5 to 13 µg/mL with no toxicity up to 100 µg/mL. Evaluation of FPAA to HIV-1 isolates resistant to reverse transcriptase or protease inhibitors yielded similar EC50 values to wild type HIV-1. Single round of virus replication, high MOI time of drug addition experiments, as well as cell-based and biochemical mechanism of antiviral action assays demonstrated FPAA inhibited the entry of HIV-1 into target cells, HIV-1 protease and reduced the cell to cell transmission of HIV-1 with EC50 values ranging from 0.5 to 7 µg/mL. Using 23 FDA-approved HIV inhibitors representative of different classes of virus inhibition, combination therapy with FPAA yielded additive to synergistic antiviral interactions. FPAA was evaluated for in vitro cytotoxicity and mechanism of cytotoxicity using in ImQuest’s ToxiSENS platform. FPAA was not toxic up to a high test concentration of 100 µg/mL to a panel of primary human cells and did not induce apoptosis, oxidative stress or affect membrane integrity. FPAA decreased PHA-stimulated PBMC proliferation when evaluated by BrdU ELISA. Macromolecular synthesis evaluation in stimulated PBMCs determined a slight decrease in RNA and protein synthesis when treated with fullerene. These studies indicate that PPAA represents a new potent anti-HIV drug candidate which could potentially be used in combination with approved anti-HIV drugs.

### 50 The Discovery of Novel Bioactive Small Molecules Targeting the Priming Complex of HIV-1

**Tracy Hartman**, Richard Guenther, Sam Yenne, Steve Peterson, Robert Buckheit, Jr., Daniel D. Sternbach

1TRANA Discovery Inc., Caryl, USA, 2ImQuest Biosciences, Frederick, USA

The next generation of antiviral therapeutics will likely target viral functions not yet exploited, such as the HIV viral priming complex. This complex is essential for activation of the reverse transcriptase enzyme and represents a therapeutic target that involves a host factor. HIV selects only human tRNA\(^{\text{Lys3}}\) for this primer. Following extensive transformation of the native conformation, HIV modifies the anticodon stem loop (ASL) into a platform for creating this primer complex. In addition to the 18 base pair duplex formed between the viral genome and the 5’end of human tRNA\(^{\text{Lys3}}\) at the viral primer binding site, there is a second region of interaction. This region has been shown to be essential for viral replication in both deletion and antisense characterization studies which validate this interaction as a target for therapeutic intervention. To discover small molecule therapeutics, a proprietary screening assay designed. After screening a diverse set of 300,000 small molecules, two unique scaffolds were identified (see below). Biochemical and structural docking experiments by NMR have confirmed specific interactions between two of the bioactive hits and the RNA complex. These experiments provided atomic level models of the RNA/RNA/small molecule complexes. Using this NMR information 18 analogs of these scaffolds were selected and tested for inhibition of viral replication in a PBMC assay. Out of the 18 derivatives tested several had good activity in a PBMC assay without concomitant toxicity. The results of the bioassay and the SAR will be presented.
51 A Novel Allosteric Small Molecule Inhibitor Of Inducible Hsp70 Reduces Dengue Virus Infection.
Matthew K. Howe1, Brandt Levitt2, Brittany L. Speer1, Timothy A. Haystead1
1Department of Pharmacology and Cancer Biology, Durham, USA, 2Department of Molecular Genetics and Microbiology, Durham, USA

The inducible protein chaperone Heat shock protein 70 (Hsp70i) is important in maintaining protein folding and cellular homeostasis. Hsp70i is utilized throughout the viral lifecycle for replication and propagation of the virus. Dengue virus, HIV, and rotavirus are a few of the viruses that exploit Hsp70i for infection and replication. However, the complete role of Hsp70i in dengue virus pathogenesis remains unclear. Previous studies have shown that Hsp70 may act as a receptor complex for virus internalization. Additionally, Hsp70 siRNA knockdown reduced dengue virus load, and Hsp70 aids in propagating the virus following internalization. Dengue virus is now endemic in over 100 countries and there are currently no approved vaccines or treatments for dengue virus infection. To date, few Hsp70 inhibitors have been identified and characterized, and their efficacy in clinical settings is unknown. We have identified a novel allosteric small molecule inhibitor of Hsp70i, HS-72, using FLECS (fluorescence linked enzyme chemoproteomic strategy). Inhibition of Hsp70i with HS-72 reduces dengue virus infection in a monocyte cell line. Additionally, HS-72 reduces binding and entry of dengue virus in monocytes. Hsp70i is expressed at low levels preceding infection, but intracellular Hsp70i expression is rapidly induced upon dengue virus infection. Furthermore, increasing Hsp70 expression prior to infection through Hsp90 inhibition, leads to an increase in dengue virus infection. This work highlights an essential role for Hsp70 in dengue virus pathogenesis and identifies a potential therapeutic anti-viral agent for dengue virus infection.

52 Comparative Analysis of Cell-based Assays to Screen Small Molecules for HIV Reactivation
David Irlbeck1, Robert Ferris1, Eugene Stewart2, Katrina Creech3, Aaron Goetz2, Kevin Brown1, Cristin Galardi1, David Favre1
1HIV Discovery Performance Unit, GlaxoSmithKline, Research Triangle Park, USA, 2Computational Chemistry, GlaxoSmithKline, Research Triangle Park, USA, 3Biological Sciences, GlaxoSmithKline, Research Triangle Park, USA

Latently-infected CD4+ T cells are a major reservoir of HIV infection. One curative strategy involves developing drugs capable of reactivating HIV expression without inducing global cell activation. To discover novel activators of latent HIV, we used three different cell-based assays to screen a representative compound set of 27,000 small molecules: 1) a human osteosarcoma cell line expressing a luciferase reporter from a Tat-independent HIV LTR promoter (HOS-LTR-Luc), 2) a combination of three well-characterized Jurkat T-cell clones harboring 1 or 2 HIV proviruses expressing the luciferase and mouse heat stable antigen (HSA) reporters (Jurkat HIV-Luc-HSA), and 3) a human primary cell latency assay whereby CD4+ T-cells from healthy donors are infected with HIV-1 expressing luciferase and HSA in the absence of T cell stimulation (Primary HIV-Luc-HSA). Hits were defined as compounds with an activation signal of >3-standard deviations above the value of DMSO treated cells and the potency and maximum response of hits were determined using dose-response curves. Hit rates ranged from 7.6% for the HOS-LTR-Luc assay to 5% for the Jurkat HIV-Luc-HSA assay and 13% for the Primary HIV-Luc-HSA assay. Boolean analysis of the hit compounds revealed subsets of compounds that were active in just one of the assays, shared activation profiles between certain pairs of assays or demonstrated a response in all three assays. Differences in activity between assays could be related to cell-specific transcription factors, Tat-(in)dependence, and/or differences in activation pathways between cell types. Following the screen of the focused compound set, we performed a high throughput screen of 1.6 million compounds using the Jurkat HIV-Luc-HSA assay and identified ~6,000 compounds (0.4%) with >2-fold activation. The potency and maximum response of these compounds was determined using dose-response curves and the tractability of these hits as potential agents for HIV curative strategies will be further investigated.
53 **AVI-7288 Provides Significant Survival Benefit for NHP Marburg Virus Infection and is Safe in Human Volunteers**

**Patrick Iversen**\(^1,2\), Alison Heald\(^1\), Travis Warren\(^3\), Jay Wells\(^3\), Pete Sazani\(^1\), Amy Shurtleff\(^3\), Lisa Welch\(^3\), Sina Bavari\(^3\)

\(^1\)Sarepta Therapeutics, Cambridge, USA, \(^2\)Oregon State University, Corvallis, USA, \(^3\)USA Army Medical Research Institute of Infectious Disease, Fort Detrick, USA

**OBJECTIVES:** Marburg virus (MARV) is a virulent RNA virus and a causative agent of viral hemorrhagic fever (VHF) in humans. In the cynomolgus macaque VHF model, death occurs 7 to 12 days post-infection. The objectives were to evaluate the therapeutic benefit from AVI-7288 and assess its safety in compliance with the Animal Rule guidance for biological threat agents.

**Methods:** Non-human Primate (NHP) Challenge: Five groups of six cynomolgus macaques per group (both male and female) were infected subcutaneously with 1,000 plaque forming units of MARV Musoke at time zero (>100X a lethal challenge). AVI-7288 was administered at 15 mg/kg intravenously (IV) once a day for 14 days with the first dose delivered in group 1 at 1 h, in group 2 at 24 h, in group 3 at 48 h, and in group 4 at 96 h post-infection. Group 5 received phosphate buffered saline vehicle IV daily at 1h. The primary endpoint was survival to day 41 post-infection. Human Multiple Ascending Dose (MAD) Safety: Five cohorts of healthy volunteers administered AVI-7288 up to 16 mg/kg IV once a day for 14 days and monitored for adverse events to assess safety.

**RESULTS:** NHP Challenge: Survival to 41 days post-infection in 83% (5/6), 83% (5/6), 100% (6/6), and 83% (5/6) of groups 1, 2, 3, and 4, respectively, compared to 0% (0/6) survival in the untreated control group.

Human MAD Safety: No significant safety concerns were observed following daily doses of AVI-7288 up to 16 mg/kg/day for two weeks.

**CONCLUSIONS:** The PMOplus™ oligomer (AVI-7288) targeting NP provides survival benefit when administered for up to 96 hours post infection from MARV lethal challenge in NHP. AVI-7288 is safe and well-tolerated in healthy human volunteers at doses up to 16 mg/kg/day. Acknowledgment: BioDefense Therapeutics (BD-Tx) is a component of the Medical Countermeasure Systems Joint Project Management Office (JPM-MCS) within the U.S. Department of Defense. The views are those of the authors and do not necessarily represent the official position of JPM-MCS or BD-Tx. Visit www.jpeocbd.osd.mil.

54 **Determination of Antiviral Effects on BKV Replication in Salivary Gland Cells**

**Liesl K Jeffers-Francis**, Raquel A Burger-Calderon, Jennifer Webster-Cyriaque

University of North Carolina at Chapel Hill, Chapel Hill, USA

**Objective:** HIV-associated salivary gland disease (HIVSGD) is among the most important AIDS oral lesions. Polyomavirus, BKV DNA was detected at high levels in HIVSGD patient saliva and replicates in salivary gland cells in vitro. The goal of this study was to investigate the effect of ciprofloxacin, cidofovir, and leflunomide on BKV replication in salivary gland cells, the primary target cells in HIVSGD. Method: Human submandibular gland cells and Vero cells were assessed in parallel. Cells were infected with BKV, drugs added 24h post infection, and assessment occurred at days 3-5 post treatment. Result: Ciprofloxacin consistently decreased BKV TAg and VP1 mRNA expression via qRTPCR by at least 50% in both cell types, and decreased TAg protein expression at days 3 and 4 as determined by immunoblot and immunofluorescence. A 2.5-4 log decrease in intracellular DNA replication and 2-3 log decrease in progeny release was detected upon ciprofloxacin treatment by qPCR. Cidofovir and leflunomide less consistently inhibited BKV gene expression, DNA replication and progeny release. Three HIVSGD clinical BKV isolates were assessed for their response to these agents in vitro. All three drugs diminished progeny release by 30-90%. Ciprofloxacin and cidofovir had minimal effect on metabolic activity and host cell DNA replication, however, some cell toxicity was detected at the protein level with leflunomide Conclusion: In conclusion, BKV replication was inhibited by all three drugs tested, with ciprofloxacin being the most effective and leflunomide the least effective. These studies provide promising therapeutic intervention for HIVSGD, a disease currently treated palliatively.
55 Assessing the Potential for Mitochondrial Toxicity of Ribonucleoside Analogs
Zhinan Jin, Jerome Deval, Julian A. Symons, Kenneth Shaw, Gary Wang, Natalia Dyatkina, Leonid Beigelman, David B. Smith
Alios BioPharma, South San Francisco, USA

BACKGROUND: Nucleoside analogs play an important role in the treatment of viral diseases. To discover safe and effective nucleoside analogs, we have established a screening paradigm designed to derisk the potential for mitochondrial toxicity of ribonucleoside analogs. We have exemplified the utility of our strategy using R1479, PSI-7851 and INX-08189, nucleoside analogs that advanced to human clinical trials for the treatment of chronic hepatitis C. R1479 (as the prodrug R1626, balapiravir) and INX-08189 (as BMS-986094) were discontinued during Phase 2 studies due to significant toxicity associated with chronic treatment. PSI-7851 advanced through Phase 3 studies as GS-7977 (sofosbuvir) and was recently granted FDA approval.

METHODS: Compounds were assessed for activity and cytotoxicity in a 3-day HCV genotype 1b replicon assay in Huh7 cells, and for cytotoxicity across a panel of six cell lines in 8-day assays. The compounds were tested for inhibition of mitochondrial protein (MP) synthesis in HepG2 cells. The corresponding nucleoside triphosphate (NTP) derivatives were examined for their potential to serve as substrates for human mitochondrial RNA polymerase (HMRP).

RESULTS: In the HCV replicon assay, all compounds displayed potent antiviral activity. R1479 and PSI-7851 were not cytotoxic (CC₅₀ >100 microM), whereas, INX-08189 demonstrated a CC₅₀ of 3.0 microM and was also highly cytotoxic against the panel of six cell lines. In contrast, R1479 and PSI-7851 did not display cytotoxicity against the panel of cell lines. The NTPs of R1479 and INX-08189 were efficiently incorporated by HMRP, but the NTP of PSI-7851 was not significantly incorporated. Further, R1479 and INX-08189 were potent inhibitors of MP synthesis whereas PSI-7851 did not inhibit MP synthesis at concentrations up to 100 microM.

CONCLUSIONS: The potential for mitochondrial toxicity of ribonucleoside analogs can be derisked through the use of HMRP-incorporation and cell based assays in the screening paradigm. We are currently applying this approach to the discovery of novel, potent purine and pyrimidine based nucleoside analogs as potential treatments for multiple viral diseases.

56 Genetic and Phenotypic Analysis of Seasonal Influenza Viruses in South Korea from 2011/2012 to 2013/2014 Seasons
Chi-Kyeong Kim, Mi-Sun Kim, Su-Jin Kim, Hyuk Chu, Jang-Hoon Choi, Joo-Yeon Lee, Soonyoung Han, Chun Kang
Division of Influenza Viruses, Center for Infectious Diseases, National Institute of Health, Korea CDC, Cheongwon-gun, South Korea

Since 2007/2008 season, the frequency of oseltamivir resistance in seasonal A/H1N1 viruses has been increased worldwide. Antiviral monitoring for emergence of drug resistant variant has been important to control and prevent of influenza. Therefore, we analyzed the genetic and phenotypic characteristics to antiviral drug resistance for seasonal influenza virus in Korea from 2011/2012 to 2013/2014 seasons. To investigate the resistance to M2 inhibitor and neuraminidase inhibitors (NAIs), we selected the seasonal influenza viruses isolated from Korea Influenza and Respiratory Virus Surveillance System (KINRESS) in two consecutive influenza seasons in Korea. The matrix protein (MP) and neuraminidase (NA) genes from these viruses were sequenced and then genetically analyzed. For phenotypic analysis, fluorometric NA inhibition assay to NA inhibitors was used and IC₅₀ was analyzed. For the genotypic analysis to amantadine, a total of 1,279 influenza A seasonal isolates were selected, of which 100% in pandemic A (H1N1) 2009 viruses and 100% in A/H3N2 were resistant during these period. All resistant viruses had substitution at amino acid 31 (S31N) that was known to be related with drug resistance. In the NA gene of influenza A (H1N1) pdm09, A/H3N2, and B viruses, none of the mutational hot spots were substituted at the level of amino acid in sequence analysis. In the phenotypic assay, all tested viruses to NA inhibitors were sensitive (0%, 0/329). NA inhibition assay showed type A influenza viruses were susceptible to NAIs than type B viruses. The IC₅₀ value to oseltamivir of both type A and B viruses were lower than those of zanamivir in the 2011/2012 season. Overall, influenza A viruses were resistant to the M2 inhibitor, but there was no resistant virus to the NA inhibitor in South Korea. However, it is still possibility to emerge the drug resistant variant with increased use of antiviral drug to treat influenza infections. Therefore, more strengthened surveillance and monitoring for emergence of resistant viruses should be consistently performed. This study was supported by the intramural fund (4834-303-210-13).
57 Abstracts

**News in the Development of 5-azacytosine-based Antivirals**

Marcela Krecmerova¹, Alice Chupikova¹, Miroslav Otmar¹, Graciela Andrei², Jan Balzarini², Robert Snoeck²

¹Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic, ²Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

In 2007, we described a new class of acyclic nucleoside phosphonates (ANPs) containing 5-azacytosine as a base moiety; among them HPMP-5-azaC and its ester produgs exerting extraordinary activity against a wide range of DNA viruses [1]. Detailed studies were focused especially to the viral infections where appropriate treatment is still very limited or not available, e.g. polyomavirus (BKV) or γ-herpesvirus infections [2,3]. The data obtained in these studies indicate that HPMP-5-azaC, cHPMP-5-azaC and HDE-cHPMP-5-azaC are the most potent and selective inhibitors of these viruses and can be attractive candidates for further clinical development. Recently, we have found that not only HPMP-5-azaC but also some other 5-azaC containing ANPs can reveal interesting activities if transformed to appropriate produgs. E.g. 1-[2-(Phosphonomethoxy)ethyl]-5-azacytosine (PME-5-azaC) is inactive in a form of a free phosphonic acid whereas in a form of bis(amidate) produg shows interesting activities against CMV and VZV. Another topic to be examined in more details are 5,6-dihydro-5-azacytosine derivatives. To increase stability of a triazine ring of 5-azacytosine compounds we work on its structural modifications which can be realized as follows: a) Derivatives substituted with phenyl and pyridyl aromatic system on a base moiety b) Compounds with 5-membered heterocycles as cytosine mimics (imidazole, triazole and thiadiazole derivatives) where 5,6-double bond of 5-azacytosine is replaced with heteroatom (e.g. sulfur atom) whose bigger bond distances to neighboring atoms could mimic the double bond. These nucleobases are more stable in comparison with 5-azacytosine. This work was supported by the Subvention for development of research organization RVO 61388963 and by the grant M200551201 by the Academy of Sciences of the Czech Republic. References: 1. Krecmerova, M. et al. J. Med. Chem. 2007, 50, 1069-1077. 2. Topalis D. et al. Antimicrob. Agents Chemother. 2011, 55, 1961-1967. 3. Coen, N. et al. J. Virol. 2013, 87, 12422-12432.

58 Abstracts

**Characterization of an HIV Entry Inhibitor Targeting a gp120-gp41 Interface**

Daniel Leaman¹, Edward Murray², PJ Klasse³, Michael Zwick¹

¹TSRI, La Jolla, USA, ²Pfizer, Sandwich, United Kingdom, ³Cornell, NY, USA

Antiretroviral drugs can control HIV-1 replication, but have led to drug resistance and a need for novel inhibitors. We have identified a low-molecular-weight HIV-1 entry inhibitor, PF-68742, that targets a novel site on Env at a post-attachment step. Mutations in the gp41 disulfide loop (DSL) region and gp120 C5 cause resistance to PF-68742, suggesting that the compound binds to a gp120-gp41 interface. However, PF-68742 enhanced infectivity of certain mutants in the gp41 fusion peptide (FP). Surprisingly, the inhibitor VIRIP, which binds to the FP, also enhanced infection of FP mutants. Partial occupancy of mutant Env by the inhibitors appears to augment fusion, whereas full occupancy blocks infection. Enhancement of FP mutants by PF-68742 and VIRIP seems to slow fusion kinetics, whereas the compounds differentially alter coreceptor interactions. Our results are consistent with a functional link between coreceptor recognition and FP insertion, which can be mediated via the DSL/C5 interface.

59 Abstracts

**Antiviral Action of the Benzodiazols Derivatives**

V Lozitsky¹, A Fedchuk¹, T Grydina¹, S Basok², L Mudryk¹, L Shitikova¹, L Socheslo¹

¹Ukrainian I.I. Mechnikov Anti-Plague Research Institute, Odesa, Ukrenia, ²A.V.Bogatsky Physic-Chemical Institute, Odesa, Ukrenia

Influenza is the most mass respiratory tract viral disease demonstrates itself in the form of the seasonal waves and epidemics. The creation of the new effective anti-influenza means as well as the instruments of their construction are the actual tasks of the contemporary medical science. The purpose of this study is to research antiviral activity of the benzodiazols’ derivatives: [3-(1H-1,3-benzodiazol-2-yl)propyl]-(methyl) amine (C-1) and 1-(1H-1,3-benzodiazol-2-yl)ethan-1-amine (C-2). Methods of the compounds activity studies in vitro on the model tissue culture of chorio-allantoic covers of 10-12-days chicken embryos (CAC) and on
the model of MDCK cell culture were used. Influenza viruses A/PR/8/34 (H1N1), A/Hong Kong/1/68 (H3N2) and avian influenza H5N3 were solved in cultural media in the presence (experiment) and in the absence of compounds (control) to a concentration of 10,000(1x10^4 log_{10}) TID50. Control and experimental samples were incubated at 37°C for 24h. The number of infectious virus in the samples was determined by titration on fragments of CAC. Also antiviral activity was determined by infecting monolayers of MDCK cells with 10-fold dilutions of viruses A/PR/8/34(H1N1), A/Hong Kong/1/68(H3N2) in presence (experiment) or absence (control) of C1 and C2. Control and experimental samples were incubated at 37°C for 72h. Presence of virus was registered in haemagglutination test. C-1 inhibited reproduction all of the strains viruses on the model tissue culture of CAC, but only slightly (A/Hong Kong/1/68 H3N2)-0,92, A/PR/8/34(H1N1) and avian influenza (H5N3)-0,75 log_{10} TID50 as compared to control). This effect was considerably higher in cells culture MDCK (A/Hong Kong/1/68(H3N2)-5,66, A/PR/8/34(H1N1)-6,0 log_{10} TID50 as compared to control). C-2 showed higher antiviral activity on the model tissue culture of CAC (A/Hong Kong/1/68(H3N2) 2,75, A/PR/8/34(H1N1)-1,08 and avian influenza H5N3-0,58 log_{10} TID50 as compared to control). These data were not much higher than in cells culture MDCK (A/Hong Kong/1/68(H3N2)-1,86, A/PR/8/34(H1N1)-2,8 log_{10} TID50 as compared to control). So studied benzodiazols derivatives show anti-influenza activity in all used models. This research was supported by STCU Grant P407

60 Synthesis and Antimicrobial Evaluation of Some Novel 1-naphtyl Acetic Acid Derivatives
Hossein Mostafavi, Elham soleymani Sani Dogjan, Peyman Zare

Faculty Of Chemistry University of Tabriz, Tabriz, Iran, Faculty of Chemistry, Tabriz, Iran, Faculty of Veterinary medicine, Tabriz, Iran

Pharmacological properties of 1-naphtyl acetic acid and amino acids derivatives prompted us to formation amide bond between amino group of different esters of amino acids and 1-naphtyl acetic acid. A series of ten 1-naphtyl acetic acid derivatives with amino acid alkyl esters linkage were synthesized and their structure confirmed by FT-IR, 1H NMR, 13C NMR and elemental analysis. Detection of biological activity of these compounds against Entobacter aerogenes ATCC 13048, Klebsiella pneumonia ATCC 700603, Escherichia coli ATCC 25922, Proteus mirabilis ATCC 43071 as (Gram-negative) bacteria and Enterococcus faecalis ATCC 29212, Staphylococcus ATCC 25952 as (Gram positive) bacteria was done by use of the paper disc diffusion method on Mueller Hinton agar (Merck). Chloramphenicol and Ciprofloxacin were standard reference antibiotics. The zone of inhibition against bacteria was measured after 24 hours at 37 °C. These compounds show low to moderate antibacterial activity.

61 Synthesis and Antimicrobial Activity of Novel Succinimides Derivatives
Hossein Mostafavi, Zahra Taghizadeh, Haedeh Mobeyen

Faculty Of Chemistry University of Tabriz, Tabriz, Iran, Faculty of Chemistry, Tabriz, Iran, Faculty of medicine, Tabriz, Iran

Imide derivatives are a valuable group of bioactive compounds showing anti inflammatory, anxiolytic, antiviral, antibacterial and antitumor properties. In spite of their wide applicability, available procedures for their synthesis limited. Among them, the dehydrative condensation of an anhydride and an amine at high temperature and the cyclization of the amic acid in the presence of acidic reagents are the typical method of choice. Seven succinimides derivatives were synthesized with this method and their structure confirmed by FT-IR, 1HNMR, 13CNMR and elemental analysis. The antimicrobial activity of synthetic compounds were determined against Gram-positive and Gram-negative bacteria. The Gram –positive bacteria were Staphylococcus aureus ATCC 25952 and Enterococcus faecalis ATCC 29212 and the Gram –negative were Enterobacter aerogenes ATCC 13048, klebsiella pneumonia ATCC 700603, Escherichia coli ATCC 25922 and Proteus mirabilis ATCC 43071. Compounds 2 and 3 show a high antibacterial activity against Gram-negative but not Gram positive bacteria. REFERENCE: 1)Kai Li, Chao Yuan et al. 2012, Tetrahedron letters, 53, 4245-4247. 3)
Modeling of Wide Spectrum of Anti-Influenza Activity Using HiT QSAR

Eugene Muratov¹,², Anatoly Artemenko¹, Ekaterina Varlamova¹, Liudmila Ognichenko¹, Stepan Basok¹, Elena Alekseeva¹, Alla Fedtchuk³, Victor Kuz’mín¹
¹A.V.Bogatsky Physical-Chemical Institute NAS of Ukraine, Odessa, Ukrenia, ²University of North Carolina, Chapel Hill, USA, ³I.I. Mechnikov Ukrainian Anti-Plague Research Institute, Odessa, Ukrenia

The goal of this study is to design novel selective anti-influenza agents by means of Quantitative Structure-Activity Relationship (QSAR) modeling of antiviral activity of the diverse set of chemical compounds. The models were developed using Hierarchic QSAR Technology (HiT QSAR) based on Simplex representation of molecular structure (SiRMS) and random forest statistical method. The set of damantane derivatives, crown and aza-crown ethers, and known antiviral drugs was tested against the following activities: 1 Inhibition of reproduction of the influenza strain H1N1 (A/PR/8/34) in experiments in CAM tissue culture (41 compounds) 2 Inhibition of reproduction of the virus strain H1N1 (A/PR/8/34) in cell culture MDCK (21 compound) 3 Inhibition of the reproduction for the strain H5N3 (54 compounds) (expressed in lgTID₅₀ and reflected suppression of viral replication in «experimental» samples to «control»). 4. Inhibition of the reproduction for the virus strain (H3N2) /Hong Kong/1/68 (34 compounds) 5. Toxicity against the Colpoda steinii culture (85 compounds) and 6. Chemical-therapeutical Index for H1N1 strain (27 compounds). We succeed to develop robust and predictive QSAR models. Then we used developed models for the virtual screening and molecular design of new anti-influenza agents with increased anti-influenza activity and selectivity, and reduced toxicity. Two compounds 1-(1H-1,3-benzodiazol-2-yl)ethan-1-amine and [3-(1H-1,3-benzodiazol-2-yl)propyl](methyl)amine has been recommended for further experimental testing. Compounds has been synthesized by chemists using condensation of 4-orthophenylenediamine with methylaminobutyric acid and S-α-alanine in an acidic medium. The results of experimental testing confirmed predicted values of antiviral activity chemical-therapeutical index, and cytotoxicity. This work is partially supported by Science and Technology Center in Ukraine (Project P407).

Synthesis and Investigation Of Potential Anti-HIV active nucleoside triphosphate (NTP) Prodrugs

Tobias Nack¹, Jan Balzarini², Chris Meier¹
¹Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany, ²Rega Institute for Medical Research, Katholieke University of Leuven, Leuven, Belgium

Nucleoside analogs are widely used for the treatment of antiviral infections and anticancer chemotherapy. A limitation of these compounds is that they have to undergo biotransformation into the corresponding NTPs via stepwise phosphorylation catalyzed by kinases. Nucleotide prodrugs represent a promising alternative to improve the biological activity of common nucleoside analogs and are valuable tools for studies regarding the nucleoside metabolism. Recently, we reported on the DiPPro approach for the bioreversible protection of nucleoside diphosphates. In contrast to the cycloSal approach, here the delivery mechanism relies on an enzymatically triggered process. Since a variety of nucleoside diphosphates with different aliphatic masking units have been synthesized and investigated, we were able to transfer this concept to NTPs. Starting with d4T (1), the nucleoside analogue is stepwise phosphorylated to the d4T diphosphate (2) using the cycloSal technology. The corresponding NTP prodrugs 3 were obtained by dicyanoimidazole mediated coupling with bis(4-aryloxybenzyl)phosphoramidites in yields up to 70%. First hydrolysis studies of the aroyl residue containing prodrugs (R=C₆H₄-4-X) in PBS buffer and porcine esterase solution revealed the successful release of NTP.

The increased lipophilicity and consequently the cell penetrating ability by using aryl residues or long aliphatic chains are paid by an increase of the stability towards enzymatic cleavage. To circumvent this problem enhanced functionalized aliphatic chains (R=(CH₂⁻na-Y) were synthesized, which could undergo an enzymatic triggered domino cleavage process.
A Single 96-Well Plate Format for Evaluation of HBV Replication in Multiple Cell Lines

Todd B. Parsley, Lu Yang, Robert W. Buckheit Jr.
ImQuest BioSciences, Frederick, USA

We developed and qualified a 96-well single plate assay to determine the relative quantities of multiple markers of HBV replication in AD38 and HepG2.2.15 cells, including intra- and extracellular HBV DNA, pre-genomic HBV RNA, HBV cccDNA, intra- and extracellular capsids, and encapsidated HBV DNA and RNA. The antiviral activity of 3TC on HBV replication in both cell lines was evaluated for development and qualification of the single plate assay. Cells were seeded into 96-well plates and treated with serial half-log dilutions of 3TC for six days. Cell culture supernatants were collected for quantification of extracellular HBV DNA and the cells were lysed and fractionated into nuclear and cytoplasmic components for evaluation of intracellular markers of viral replication. Evaluation of the effect of 3TC on multiple HBV nucleic acid markers in both HepG2.2.15 and AD38 cells from a single 96-well plate demonstrated a dose-dependent reduction in extracellular HBV DNA, cytoplasmic HBV DNA and cccDNA in both cell lines. EC50 values for inhibition of extracellular and cytoplasmic HBV DNA accumulation were lower in the AD38 cell line than in the HepG2.2.15 and EC50 values for inhibition of extracellular DNA were lower than those for cytoplasmic DNA in both cell lines. A dose-dependent reduction in accumulation of cccDNA was also observed in both cell lines with relative cccDNA levels reduced to 50% of untreated cells in HepG2.2.15 cells and to undetectable levels in AD38 cells at the high test concentration (10μM). Reduction of pgRNA transcription by 3TC was non-specific in both cell lines. Analysis of the activity of 3TC using the assay format yielded results consistent with the inhibition of HBV replication by an inhibitor of reverse transcription. The reduction in cccDNA accumulation was consistent with the inducible nature of HBV replication in AD38 cells and the chronic infection of the HepG2.2.15 cells. Experiments to determine the relative quantity of HBV capsids and encapsidated nucleic acids within the context of the 96-well plate assay are ongoing. The assay is also being developed to incorporate the use of HepRG cells, which express the HBV receptor (sodium taurocholate cotransporting peptide) and to include the evaluation of compounds on early phases of replication.

Using Yeast-based Platform for Discovery of Drugs Against Dengue Host Factors

Kunj Pathak, Daniel Engel
Microbiology, UVA, Charlottesville, USA

Dengue fever and its complications represent one of the world’s most important emerging health threats with about 2.5 billion people, or half of the world’s population, at risk. RNA viruses like Dengue virus (DENV) rely on different host proteins to accomplish robust multiplication in their hosts. Session and co-workers (2009) identified over 300 potential dengue virus host and restriction factors through a high throughput RNAi screen in a Drosophila cell line. One of the host factor Sec61B is critical in the classical co-translational pathway. A commercial available small compound library of 20,000 drugs was tested for their effect on sec61 overexpressing yeast. Around 1500 compounds were found to re-repress sec61p slow growth phenotype. Further testing of the compounds in triplicates resulted into 169 anti host sec61p candidates. We further tested these compounds for their effect on dengue viral multiplication. Currently, three lead compounds from the above screen are being rigorously tested in different viral and cellular function assays.
New 1-Phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxamides Inhibit Hepatitis C Virus Replication and Suppress the Expression of Cyclooxygenase-2
Sveva Pelliccia¹, Dinesh Manvar², Giuseppe La Regina¹, Johan Neyts³, Neerja Kaushik-Basu², Romano Silvestri¹
¹Sapienza University, Roma, Italy, ²New Jersey Medical School, Newark, USA, ³Katholieke Universiteit Leuven, Leuven, Belgium

New pyrazolecarboxamide derivatives were synthesized as anti-hepatitis C virus agents, and the mechanism of inhibition was investigated. The most active compounds inhibited the subgenomic HCV replicon 1b in Huh 5-2 cells at EC₅₀ values between 5 and 8 µM and displayed an even higher potency against the infectious Jc1 virus in infected cells. Hit compound 14 did not target HCV NS5B or HCV IRES mediated translation, but mediated its anti-HCV effects by strongly suppressing HCV-associated dysregulation of COX-2, exhibiting an IC₅₀ of 3.2 µM in COX-2 promoter-linked luciferase reporter assay and reducing expression of endogenous COX-2 protein in a dose dependent manner. Notably, compound 14 did not show to be cytotoxic at higher concentrations (CC₅₀ >154 µM, SI = 23). Our data thus suggest that the pyrazolecarboxamide derivatives function as anti-HCV agents through targeting of COX-2 at both the transcriptional and translational levels. These results provide a strong basis for hit optimization of this new chemical class of HCV inhibitors (Chart 1).

Brincidofovir (BCV, CMX001) and Acyclovir (ACV) Are Additive Against Cytomegalovirus (CMV) In Vitro
Dean W Selleseth¹, Phiroze B Sethna¹, Mark N Prichard², Randall Lanier¹
¹Chimerix, Inc., Durham, USA, ²University of Alabama, Birmingham, USA

BACKGROUND: Brincidofovir (BCV, CMX001) is an orally bioavailable, lipid acyclic nucleoside phosphonate that is converted intracellularly into the active antiviral cidofovir diphosphate (CDV-PP). The broad spectrum antiviral activity of BCV against all five families of double-stranded DNA (dsDNA) viruses pathogenic to humans led to the design of the Phase 3 SUPPRESS trial of BCV for prevention of CMV and other dsDNA viruses in hematopoietic cell transplant (HCT) recipients. Previous studies have shown synergistic activity of ACV and BCV against herpes simplex virus. As ACV is commonly administered in HCT recipients, the in vitro activity of combination ACV and BCV against CMV was explored.

METHODS: Human embryonic lung fibroblast cells (MRC-5) were infected with CMV strain AD169 at an MOI of 0.006 plaque-forming units (PFU) per cell. Serial dilutions of BCV or ACV alone and in combination (0.03 nM to 4 nM for BCV, 12.5 µM to 200 µM for ACV) were added to the CMV-infected cells. Intracellular CMV DNA was harvested after a 7-day incubation and viral DNA copy number was determined by quantitative PCR.

RESULTS: BCV was highly active against CMV with a mean EC₅₀ of 0.00036 µM. ACV was approximately five orders of magnitude less active against CMV, with a mean EC₅₀ of 33 µM. Combinations of BCV and ACV were additive against CMV.

CONCLUSIONS: These data are consistent with prior studies of the in vitro antiviral activity against CMV for the individual agents, BCV and ACV. Although ACV plasma exposure following oral Valtrex can be relatively high (e.g., ACV Cmax ~25 µM for 1000 mg dose), it has generally proven ineffective in prevention of CMV reactivation in randomized trials. By comparison, the Cmax produced by 100 mg BCV (Cmax ~0.5 µM) is more than 100-fold higher than the EC₅₀ for CMV, consistent with the significant decrease in rates of CMV reactivation demonstrated in the Phase 2 dose-ranging trial. The results from this series of in vitro experiments suggest that co-administration of ACV is unlikely to have a significant impact on the rate of CMV reactivation in the setting of prophylactic use of BCV.
Determination of Hepatitis C Based Using the Anti HCV and HCV Ag
Nafija Serdarevic
University of Sarajevo, Sarajevo, Bosnia and Herzegovina

The aim of this study was to confirm the diagnosis of hepatitis C by determination of Anti HCV and HCV Ag. The study included 120 patients of whom 70 men and 50 women who were hospitalized at the University Clinical Centre in Sarajevo or the ambulant treated. The determination of HCV Ag and Anti HCV was done using Chemiluminescent Microparticle immunoassay (CMIA) (ARCHITECT). Total 26 patients had HCV Ag negative value (0.16 ± 0.36 fmol / L) and Anti HCV positive value (6.0 ± 5.54 S / CO). The current study, 94 patients had HCV Ag positive value (3092.48 ± 351.54 fmol / L) and Anti HCV positive value (12.61 ± 3.54 S / CO). The Abbott Architect HCV antigen assay showed a specificity of 100 percent. The intra- and interassay coefficients of variation ranged from 3.6 to 8.0 percent and from 4.7 to 9.5 percent, respectively. Except for HCV genotype 2 isolates, the analytical sensitivity was always less than 10 fmol core antigen/L, corresponding to approximately 500 to 3,000 IU of HCV RNA/mL. Linearity was guaranteed throughout the dynamic range (10 to 20,000 fmol/L). Active HCV infection, either acute or chronic, is characterized by the presence of HCV antigen analogous to HBs Ag in acute HBC infection. The negative antibody test does not rule out an HCV infection in early incubation phase. HCV antibodies are useful as an indicator of past HCV infection and do not indicate current viraemia or elimination of virus from patient. The presence of HCV RNA in the peripheral blood is the most marker of HCV replication. The patients who has positive value HCV Ag and Anti HCV have Hepatitis C infection. The negative value of HCV Ag and positive value of Anti HCV means past infection or non –specific result. Architect HCV antigen assay proved to be a specific, reproducible, highly sensitive, and clinically applicable test format that will find a place in virological HCV diagnostics.

In Vitro Selection of Brincidofovir-Resistant and Cidofovir-Resistant Human Adenovirus
Phiroze Sethna, Andrew Bae, Dean Selleseth, Randall Lanier
Chimerix Inc., Durham, USA

BACKGROUND: Brincidofovir (BCV, CMX001) is an orally bioavailable lipid acyclic nucleoside phosphonate which is converted intracellularly to the active antiviral cidofovir diphosphate (CDV-PP). BCV shares the broad-spectrum antiviral activity of CDV against all five families of dsDNA viruses which cause disease in humans, including adenoviruses (AdV). The 50 to 500-fold improved in vitro activity of BCV vs CDV is likely due to efficient transport of circulating BCV across the cell membrane, resulting in higher intracellular concentrations of CDV-PP. Mutations in the AdV DNA polymerase gene have been reported to impart resistance to cidofovir (CDV). Since the active antiviral is qualitatively the same for BCV and CDV, but quantitatively different, sequence changes in BCV-resistant and CDV-resistant viruses selected under identical conditions were compared.

METHODS: A laboratory strain of human adenovirus species C (AdV 5) was passaged in A549 cells in the presence of increasing concentrations of BCV or CDV for more than five months (15 passages). Every five passages the AdV polymerase gene sequence and EC50 of passaged virus for BCV and CDV were determined using standard methods.

RESULTS: Virus following the final passage with BCV or CDV exhibited a 4 to 8-fold increase in EC50 versus wild-type virus. Genotyping identified three amino acid changes in the AdV polymerase sequence: T87I and V303I in BCV-passaged virus and T1150I in CDV-passaged virus. These viruses were not overtly growth impaired compared to the parent AdV strain based on DNA levels after identical growth periods. One of the changes (V303I) was reported previously for CDV resistance in AdV5 by Kinchington. The other two mutations have not been reported and suggest involvement of multiple regions of the AdV pol in determining resistance to these agents.

CONCLUSIONS: Different mutation patterns were detected in resistant AdV isolates selected by BCV and CDV, although one of two mutations selected by BCV had been previously reported for CDV. The reasons for the observed difference in mutations selected could include higher levels of CDV-PP in BCV-treated cells, stochastic selection or other unidentified causes. This is the first report of selecting BCV-resistant AdV in cell culture.
**Abstracts**

**70**  
α-Pyranone Carboxamide: A New Scaffold Optimization as Possible Anti-HCV Agent  
Ashoke Sharon¹, Ananda K Konreddy¹, Massaki Toyama², Wataru Ito², Masanori Baba²,  
Chandralata Bal¹  
¹Department of Applied Chemistry, Birla Institute of Technology, Ranchi, India, ²Center for Chronic  
Viral Diseases, Kagoshima University, Kagoshima, Japan  
The appearance of drug resistance and side effects imposes the requirement for the development of new  
scaffolds as direct acting antivirals to show alternate mechanism. Herein, we present the discovery and  
synthetic optimization of new scaffold based on α-pyranone carboxamide as potential anti-HCV agents.  
A detail structure activity relationship (SAR) was explored with several newly synthesized compounds  
(approx 85 analogs). The promising compounds had shown EC50 ranging from 0.11 to 0.40 µM. One of  
the compound demonstrated potential anti-HCV activity with EC50 of 0.18 µM in cell based HCV replicon  
system with lower cytotoxicity (CC50 > 20 µM) and provided a new scaffold for anti-HCV drug development.  
Further optimization based on motif S1, S2, S3 and S4 (Figure 1) and biochemical characterization are in  
progress to elucidate its possible mode of action. (AS and CB acknowledge to Department of Science &  
Technology (DST), and UGC for research grant to establish initial infrastructure for anti-hepatitis research.  
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**71**  
In-vitro and In-vivo Study of Lamivudine Sustained Release Tablet Using  
Modified Pea Starch  
Akhilesh Vikram Singh  
Department of Materials Engineering, Indian Institute of Science, Bangalore, India  
The present investigation concerns with the development of sustained release (SR) tablets of lamivudine  
using cross-linked pea starch. The cross-linked pea starch was synthesized with phosphorous oxy-chloride in  
basic pH medium. The cross-linked pea starch was tested for acute toxicity and drug-excipient compatibility  
study using DSC technique. Lamivudine SR formulations were evaluated for physical characteristics  
(hardness, friability, % drug content and weight variations), and in vitro release study. The release study  
pattern of optimized formulation exhibited zero order kinetic and the release mechanism followed  
combination of diffusion and erosion process. There was a significant difference in the pharmacokinetic  
parameters (Tmax, Cmax, AUC, Vd, T1/2 and MDT) of the optimized formulation as compared to the  
marketed conventional tablet Lamivir®. The in vitro and in vivo study of the optimized formulation revealed  
that the cross-linked pea starch in the CR tablets rendered the drug to be released in a sustained manner.
A Convergent Synthesis of Anti-HBV Agent, FMCA and Its Prodrug (FMCAP)

Uma S. Singh1, Ram C. Mishra1, Ravi Shankar1, Masaya Sugiyama1, Rajgopal Govindarajan1, Brent Korba3, Yasuhiro Tanaka2, Chung K. Chu1

1The University of Georgia, College of Pharmacy, Athens, USA, 2University Graduate School of Medical Sciences, Nagoya, Japan, 3Georgetown University Medical Center, Washington, USA

FMCA is the pro-drug of the 2'-fluoro-6'-methylene-carbocyclic adenosine (FMCA), a potent and selective inhibitor of wild type as well as drug resistant hepatitis B virus (HBV) mutants. FMCA showed excellent antiviral activity against both adefovir resistant and lamivudine double (rtL180M/rtM204V) mutants in vitro. Furthermore FMCA demonstrated superior characteristic in vitro against lamivudine/entecavir triple resistant mutant (L180M+S202T+M202V) in comparison to lamivudine and entecavir. FMCAP demonstrated greater than 12-fold increase of anti-HBV activity. The preliminary in vivo study in chimeric mice harboring the triple mutant, FMCAP effectively reduces HBV viral load while entecavir was ineffective on viral load. FMCA has also been studied for the release of lactic dehydrogenase for potential mitochondrial toxicity which was found no significant increase in toxicity of FMCA. These promising results prompt FMCAP for further preclinical studies as anti-HBV agent. Consequently, scalable synthesis of FMCA and FMCAP was required to support the preclinical studies. Initially, the FMCA was synthesized in 22 steps that was a very lengthy and a tedious process. Here, we present a new convergent approach with efficient and scalable synthesis of FMCA, in 14 steps. Highly efficient methodology for a stereospecific production of the versatile carbocyclic key intermediate, D-2'-fluoro-6'-methylene cyclopentanol, has been developed via (-)-2-azabicyclo[2.2.1]hept-5-en-3-one. The utility of D-2'-fluoro-6'-methylene cyclopentanol is demonstrated in the preparation of FMCA.

Properties of Novel Substances Based on Plant Flavonoids and Mechanisms of Their Antiviral Activity

D.B Starosyla1, I.V. Gomolyako2, M.O. Platonov3, O.V. Vasylchenko1, M.Yu. Obolenskaja3, Yu. Porva1, S.L. Rybalko1, V.P. Lozitsky1

1L.V.Gromashevsky Institute of Epidemiology and Infectious Diseases of NAMS of Ukraine, Kyiv, Kiev, 2The O.O.Shalimov Nationality Institute of Surgery and Transplantation NAMS of Ukraine, Kyiv, Kiev, 3Institute of Molekular Biology and Genetics of NAS of Ukraine, Kyiv, Kiev, 4I.I.Mechnikov Ukrainian Anti-Plague Research Institute of the Ministry of Health of Ukraine, Odessa, Odessa,

The thesis deals with the analysis of the properties and mechanisms of antiviral activity of 7,3'-dimetoxyquercetine (DMQ) as the principal component of Proteflazid plant-derived composition and the minor synthetic component 5,7,3',4'-tetrametoxyquercetine (TMQ). The antiviral activity of the substances under study was assessed in vitro and in vivo in modeled experimental virus infections, namely influenza, herpes types 1, 2, hepatitis C. The experimental model of the generalized herpes infection with low doses of virus has been elaborated. Infection at low doses allows for more active virus replication in various target organs (brain, liver, heart, spleen, kidneys, lungs) indicating dissemination of virus and generalized herpetic infection with such manifestations as meningoencephalitis, hepatitis, myocarditis, pneumonia, nephritis, splenitis. DMQ and TMQ used both in prophylactic and therapeutic schedules inhibited effectively such generalized herpetic infection. Novel data on the anti-influenza mechanisms of DMQ related to neuraminidase inhibition have been provided. Computed modeling and docking techniques demonstrated DMQ binding to the active center of the enzyme hindering neuraminidase activity. The carbohydrate component of DMQ is important for the stability of DMQ-neuraminidase interaction. For the first time, DMQ as flavonoid component of Proteflazid has been shown to induce interferon (IFN) and up-regulate protein kinase (PK), 2’5’-oligoadenylate synthase (2’5’ OAS) and RNAse L expression with relative decrease of 2’5’ OAS up-regulation. The method for classifying IFN inducers according to the relative expression of PK, 2’5’ OAS and RNAse L genes has been elaborated and patented.
Hepatitis B Virus Replication is Blocked by N-hydroxyisoquinolininedione Inhibitors of the Viral Ribonuclease H Activity

John E Tavis¹, Catherine Cai¹, Elena Lomonosova¹, Eileen Moran¹, Xiaohong Cheng¹, Fabrice Bailly², Philippe Cotelle², Marvin J Meyers¹
¹Saint Louis University, Saint Louis, USA, ²University of Lille, Lille, France

Nucleos(t)ide analog drugs profoundly suppress Hepatitis B Virus (HBV) replication but rarely cure the infection, so therapy is essentially life-long. The nucleos(t)ide analogs often push HBV to the brink of extinction, so it may be possible to eradicate HBV by further suppressing HBV replication using new drugs together with the nucleos(t)ide analogs. The HBV ribonuclease H (RNAseH) is a logical drug target because it is the second of only two viral enzymes that are essential for viral replication, but it has not yet been exploited due to difficulty in establishing screening assays. We recently developed a low throughput screening pipeline to assess inhibition of the HBV RNAseH activity and viral replication to support anti-HBV RNAseH drug discovery. Here, we screened 23 N-hydroxyisoquinolininedione compounds for anti-HBV RNAseH activity. Nine compounds had detectable activity against either genotype C or D HBV RNAseH in biochemical studies, but activity was only marginal for 8 of these screening hits. Compound #1 (N-hydroxyisoquinolininedione, 2) was the best hit with an IC50 of ~30 µM vs. the HBV RNAseH and an EC50 of 4.2 µM against HBV replication in cell culture. It preferentially suppressed accumulation of the viral plus-polarity DNA strand, indicating that replication inhibition was due to suppression of viral RNAseH activity in cells. Counter-screening indicated that compound #1 had modest activity against human RNAseH1. It had CC50 values of 75 and 87 µM by MTT and cell rupture assays, respectively, yielding a TI of ~18. Therefore, compound #1 inhibited HBV replication by blocking the viral RNAseH activity. The EC50 value was 7-fold lower than the IC50, possibly due to cellular retention or metabolism of the compound, or to higher affinity for the native enzyme compared to the recombinant enzymes used for screening. A low resolution structure-activity relationship (SAR) was found that strongly implies the N-hydroxyisoquinolininediones will have different SARs for the HBV and HIV RNAseHs. These studies provide a foundation for development of more effective RNAseH inhibitors of HBV replication.

Fitness and Virulence of a Coxsackievirus Mutant that Can Bypass the Need for Host Cell Factor PI4KIIIβ

Hendrik Jan Thibaut¹,², Hilde M. van der Schaar¹,³, Kjerstin H.W. Lanke³, Pieter Leyssen², Martin Andrews⁴, Johan Neys², Frank J.M. van Kuppeveld¹,³
¹University of Utrecht, Utrecht, Netherlands, ²University of Leuven, Leuven, Belgium, ³Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, ⁴Galapagos BV, Mechelen, Belgium

RNA viruses can rapidly mutate and acquire resistance to drugs that directly target viral proteins. Hence there is a growing interest in the development of antiviral drugs that target host factors critical for replication, since these are unlikely to mutate in response to therapy. The host factor phosphatidylinositol-4-kinase IIIβ (PI4KIIIβ) is essential for replication of enteroviruses, a group of medically important RNA viruses including poliovirus, coxsackievirus, rhinovirus, and enterovirus 71. We identified a novel potent PI4KIIIβ inhibitor (Compound 1) that inhibits the kinase not only in vitro and in intact cells, but also showed protective activity against coxsackievirus-induced pancreatitis in mice (Van der Schaar et al., 2013). However, a lengthy selection process in cell culture resulted in the emergence of a compound-resistant coxsackievirus mutant that no longer depended on PI4KIIIβ. The aim of this study was to explore whether this mutant virus would have the fitness to establish a drug-resistant virus population. First, we tested whether the mutant would be outcompeted by wildtype (wt) virus in a competition assay. Within a mixed population (ratio wt to mutant of 1:1), the mutant was not outgrown by the wt virus during 10 consecutive passages. Next, we compared the pathogenicity of the mutant to that of wt virus in the pancreatic mouse model. The mutant proved as virulent as wt virus in mice, even in the presence of the PI4KIIIβ inhibitor. Collectively, these findings indicate that coxsackievirus can bypass the need for an essential host factor and can acquire resistance to a host-targeting compound. The mutant coxsackievirus has the fitness to establish a resistant population with a similar virulence as wildtype virus. However, given the lengthy selection process in vitro, it remains questionable whether this mutant would emerge during a short-acting treatment of these acute infections in infected patients.

75 76
Discovery of Novel Acylguanidine-based Small Molecules that Block Influenza A M2 Ion Channel Activity and Drug-resistant Virus

Ian Tietjen1, Scott C Miller1, Daniel C Kwan1, Brent Johnson2, Hannah E Boycott1, Doug Chou1, David D Busath2, David Fedida1

1University of British Columbia, Vancouver, Canada, 2Brigham Young University, Provo, USA

The recent emergence of drug-resistant Influenza A viruses highlights an urgent and unmet need for new antivirals. Here we describe a series of novel acylguanidine-based small molecules that are potent inhibitors of the M2 viral ion channel, as measured by whole cell patch clamp electrophysiology. The lead molecule, SM111, inhibits wild-type M2 with an IC50 of 0.2µM, making it more potent than the established M2 inhibitors amantadine or hexamethylene amiloride (HMA) in this assay (respective IC50s = 0.6 and 1.3µM). SM111 is also less cytotoxic than HMA (respective CC50s = 130 and 16µM; respective Selectivity Indices (SIs) = 650 and 12.3) with weaker blockade of hERG cardiac ion channel (respective IC50s = 1.5 and 3.2µM; SIs = 16.0 and 1.2). SM111 also competes with amantadine for blockade of M2. A structure-activity-relationship study further identifies an SM111 derivative called SM122 that blocks adamantane-resistant M2 with an S31N mutation (IC50 = 70+/-10µM). Interestingly, the activity of SM122 on M2 blockade is dependent on distinct M2 sequence polymorphisms not associated with adamantane resistance. Finally, we show that these molecules block wild-type and/or adamantane-resistant Influenza A virus propagation in vitro (e.g., EC50 for SM122 against adamantane-resistant virus = 2.4µM). Our study emphasizes the use of non-adamantane chemical scaffolds to develop novel Influenza A ion channel inhibitors and antivirals.

An Enterovirus 71 Mouse Model with Central Nervous System Involvement

Aloys Tijsma, Hendrik Thibaut, David Franco, Johan Neyts
Rega Institute for Medical Research, Leuven, Belgium

To aid in the development of antivirals against enterovirus 71 (EV71), a robust small animal model with CNS involvement is urgently needed; Current models make use of suckling mice and in which neurotropism is mostly not observed. We passaged an EV71 clinical isolate five times in the brain of one day old mouse pups, after which the virus was passaged twice on RD cells. Next six week old Severe Combined Immune Deficiency syndrome (SCID) mice were inoculated i.p. with 2.4*10^5 PFU of either the mouse-passaged or the parent EV71 isolate. In mice infected with the passaged strain; first signs of disease (weight loss, coordination and balancing problems and paralysis of the hind legs) developed by day five post infection and progressed rapidly. Mice in this group were on average euthanized for ethical reasons on day 7 post infection. In SCID mice that had been infected with the wild type isolate, the disease evolved much more slowly and these mice were on average euthanized on day 15 post infection. Viral RNA of the mouse-adapted strain was detected (by means of RT-qPCR) in large quantities in the brain and spinal cord whereas in other tissues much lower levels were measured. In the CNS in particular motor neurons stained positive for EV71 antigens. Unlike the mouse adapted strain, the parent isolate replicated during the first days after inoculation preferentially in the lungs but was at later times, when neurological symptoms developed, also detected in the brain. The genomes of the parent and mouse adapted viruses were sequenced. Only one missense mutation (resulting in amino acid substitution V135I in VP2) was found in the entire coding region. In SCID mice that had been infected with the wild type isolate, the disease evolved much more slowly and these mice were on average euthanized on day 15 post infection. Viral RNA of the mouse-adapted strain was detected (by means of RT-qPCR) in large quantities in the brain and spinal cord whereas in other tissues much lower levels were measured. In the CNS in particular motor neurons stained positive for EV71 antigens. Unlike the mouse adapted strain, the parent isolate replicated during the first days after inoculation preferentially in the lungs but was at later times, when neurological symptoms developed, also detected in the brain. The genomes of the parent and mouse adapted viruses were sequenced. Only one missense mutation (resulting in amino acid substitution V135I in VP2) was found in the entire coding region. In SCID mice that had been infected with the parent strain, EV71 that was recovered from the lungs carried a valine at position 135, whereas the virus that was isolated from the brain of these mice had an isoleucine at this position. Thus distinct EV71 sub-populations can be present within a single organism and amino acid 135 in VP2 is a key determinant for EV71 neurotropism in mice. The exact role of this amino acid in tissue tropism and pathology is currently being investigated. The EV71 SCID mouse model is well suited for and will now be employed to assess the efficacy of inhibitors of EV71 replication.
79 Mitochondrial Biogenesis and Respiration as Sensitive Indicators for Nucleoside Analog Toxicity
Yili Xu, Adrian S. Ray, Brian Schultz, Roman Sakowicz, Joy Y. Feng
Gilead Sciences Inc., Foster City, USA

BACKGROUND & AIM: Nucleotide inhibitors (NI) play central roles in antiviral therapy. However, some NI have been associated with toxicities putatively caused by inhibition of mitochondrial DNA polymerase gamma, mitochondrial RNA polymerase or the perturbation of natural nucleotide pools. In this study, we assessed the impact of NI associated with clinical toxicity on mitochondrial biogenesis and mitochondrial respiration in whole cells in vitro.

METHOD: Mitochondrial biogenesis was measured using in-cell ELISA to measure the levels of a mitochondrially-encoded protein [complex IV or cytochrome c oxidase 1 (COX1)] and a chromosomally-encoded protein [complex II or succinate dehydrogenase (SDHA)] simultaneously. Mitochondrial respiration was measured by oxygen consumption rates for basal respiration, maximal respiration, and spare respiration capacity using a Seahorse XF-Analyzer.

RESULTS: Decreased mitochondrial respiration was a more sensitive indicator of mitochondrial injury than either ATP levels or cell count. The effects of NI on mitochondrial respiration correlated with inhibition of mitochondrial protein synthesis. We were able to categorize the mechanisms of toxicity of NI into two distinct groups: compounds specifically toxic to mitochondria (including R1479 and ddC) and compounds impairing both mitochondrial and cellular targets (including FIAU, AZT, BMS-986094, and NM107).

CONCLUSION: This study demonstrated that effects on mitochondrial biogenesis and respiration are sensitive indicators of the potential for NI to cause toxicity. Coupled with findings from biochemical studies with isolated enzymes, these studies provide insights into the mechanisms for toxicities caused by some NI.

80 A Leading DAA ZN2007 as HCV NS3 Inhibitor with Excellent Activity and Safety by SAR Optimization for Clinical Study
Zheng-Yun James. Zhan1,2, Guoyan Zhang2, Hua Yan1, Xianjing Yu2
1AB Pharma Ltd., Shanghai, China, 2Zannan SciTech Co., Ltd., Shanghai, China

BACKGROUND: It has been intensively studied for discovery of new hepatitis C virus (HCV) inhibitors since HCV was reported in 1989. So far, we have developed several competitive HCV NS3 inhibitors with excellent potency and safety by in vitro evaluation, and our goal is to discover a leading HCV protease inhibitor by screening different kinds of new anti-HCV compounds in preclinical studies.

RESULTS: In comparison with different kinds of reported HCV-NS3 inhibitors, we have developed several kinds of new macro-heterocyclic based structure of HCV-NS3 inhibitors by Structure Activity Relation (SAR) and in vitro evaluation, and finally discovered a leading HCV NS3 inhibitor with high antiviral activity, excellent selectivity (ie, direct acting antiviral, DAA). Here we disclose preclinical results of the oral toxicity and PK studies of the leading HCV-NS3 inhibitor ZN2007 with pan-genotypic activity (EC50: 0.1-5nM for Ia, Ib, IIA and IVA, respectively) and PK in rats, dogs and monkeys, and there were no any serious drug-related toxicity and no adverse events observed in the bioavailability study in dogs with compound ZN2007 by comparison of the venous injection and formulated tablet, respectively. Furthermore, several kinds of stable tablet formulations were developed and evaluated with very good bioavailability (30-40% in dogs) for ZN2007. Finally, an excellent tablet formulation was developed with high bioavailability and excellent safety for clinical studies.

CONCLUSIONS: By optimization of the SAR and in vitro evaluation, a leading DAA HCV-NS3 inhibitor ZN2007 was well developed with excellent potency, safety, PK and bioavailability as a stable tablet drug for clinical Phase I study in USA in 2014. Our goal is to develop a leading anti-HCV combination therapy with both of our well optimized HCV NS3 inhibitor ZN2007 and another picomolar pan-genotypic NS5A inhibitor.
MEDICINAL CHEMISTRY
Chair(s): Chris Meier, Ph.D.
9:15 am – 10:15 am
BALLROOM A

Michela Cancellieri1, Marcella Bassetto1, Gilda Giancotti1, Pieter Leyssen2, Johan Neyts2, Andrea Brancale1
1Cardiff University, United Kingdom, 2University of Leuven, Belgium

Chikungunya virus (CHIKV) is a mosquito-borne Alphavirus that is re-emerging worldwide and that causes an acute illness characterized by fever and arthralgia. There is no vaccine or antiviral therapy available, and only very few selective inhibitors of virus replication have been reported so far. The non-structural protein 2 (nsP2) is an essential multi-functional viral protein with cysteine-protease activity that cleaves the nsPs polyprotein precursor into the four mature nsPs. Using computer-aided approaches on a homology model of the CHIKV nsP2 protein that we developed, an inhibitor of CHIKV replication with activity in the low µM range was identified. A series of novel derivatives, previously designed to explore the biological activity of this molecule, led to the synthesis of new structural analogues with improved chemical properties and activity at low µM concentration. In this presentation, we will show a new series of compounds that was designed, based on computational analyses on the CHIKV nsP2 crystal structure, for further optimization of the first hits. The envisaged modifications were aiming to improve the stability, solubility and chemical features of the original compounds. We will discuss the rational and the chemical approach for the synthesis, as well as the SAR associated with this new series of anti-CHIKV derivatives.

83 Development of the Central Nervous System (CNS)-Targeting Protease Inhibitors for Treating HIV-Associated CNS Disorders
Hiroaki Mitsuya1,2, Masayuki Amano2, Miguel Gómez2, Manabu Aoki2, Hironori Hayashi2, Sofiya Yashchuk3, Debananda Das1, Arun Ghosh3
1Exp Retrovirol Sec, Nat’l Cancer Inst, NIH, Bethesda, USA, 2Depts of Infect Dis and Hematol, Kumamoto Univ Sch Med, Kumamoto City, Japan, 3Depts of Chem and Med Chem, Purdue Univ, West Lafayette, USA

Combination antiretroviral therapy (cART) has significantly reduced the incidence of primary HIV encephalopathy. However, the prevalence of CNS disorders such as HIV-associated neurocognitive disorder (HAND) is increasing as a result of successful cART-induced prolonged patient survival. Most currently FDA-approved anti-HIV drugs including protease inhibitors (PIs) only poorly penetrate into CNS, allowing continuing CNS infection by HIV that evolves independently over time. Moreover, cART is less effective in lowering virus replication in CNS than in circulation. We have focused on the design and synthesis of non-peptidyl PIs that potentially penetrate CNS and are potent against HIV variants resistant to the currently approved PIs including darunavir (DRV). We identified various non-peptidic PIs such as GRL-04810 and GRL-05010, which contain a structure-based designed privileged nonpeptidic P2 ligand, bis-tetrahydrofuranylurethane or its related moiety with a mono- or di-fluorine moiety and exert highly potent activity against a wide spectrum of multi-drug-resistant HIV variants including highly DRV-resistant variants with EC50 values of subnanomolar concentrations with minimal or least in vitro cytotoxicity (CC50 values of 20 µM specificity indexes of 13,500 to 147,500). Such novel PIs have favorable lipophilicity profiles as determined with the partition (logP) and distribution coefficients (logD) and appear to have a greater advantage in crossing the blood-brain-barrier (BBB) than the currently approved drugs as determined with a novel in vitro BBB permeability assay that incorporates a triple culture of rat-derived astrocytes, pericytes and monkey-derived endothelial cells. Thus, the novel PIs discovered in the present work exert potent activity against a wide spectrum of HIV variants in vitro and the data suggest that fluorine moieties added may well enhance their penetration across BBB into CNS in individuals with HIV infection.
84 Synthesis and Characterization of Biologically Active Nucleoside Triphosphate Prodrugs

Tristan Gollnest1, Jan Balzarini2, Chris Meier1
1University of Hamburg, Hamburg, Germany, 2Katholieke Universiteit Leuven, Leuven, Belgium

Over the last decades a variety of nucleosides were applied in antitumor and antiviral therapy. However, for example 2',3'-dideoxy- or 3'-modified-nucleosides are limited in their efficiency due to the necessity of intracellular phosphorylation steps by kinases. If the biotransformation into the corresponding NTP occurs insufficiently, the antiviral efficacy is very low. Due to their polarity the application of negatively charged phosphorylated nucleosides is not possible. An option to circumvent this problem is the use of lipophilically masked phosphorylated nucleoside analogs, which are able to penetrate the cell membrane and provide the corresponding nucleotide by e.g. enzymatic hydrolysis. The cycloSal-prodrug system was developed for nucleoside monophosphates and was also applied to different nucleoside analogs. Recently, we reported on a convenient approach called DiPPro-concept for the delivery of nucleoside diphosphates. Therefore, we approached now the development of nucleoside triphosphate prodrugs. A number of d4TTP prodrugs with different aliphatic masking units have been synthesized. Furthermore, a variety of nucleoside analogs have been investigated. The synthesis was achieved by using acceptor-substituted cycloSal phosphate triesters for the preparation of nucleoside diphosphates. Finally, the prodrug was formed by dicyanoimidazole mediated coupling of the corresponding phosphoramidites with NDP in yields up to 50%. Chemical hydrolysis studies in PBS buffer and enzymatic cleavage in CEM/0 cell extract showed the successful release of NTP.

85 Chemical Optimization of Novel Inhibitor Classes Selectively Targeting PI4KIIIbeta: a Host Lipid Kinase Crucial for Enterovirus Replication

J. Brad Shotwell, Shihyun You, Lisa Shewchuk, Liping Wang, Scott Dickerson, Ping Xiong, Rich Peterson, Jeff Gobel
GlaxoSmithKline, Research Triangle Park, USA

The replication of multiple enteroviruses including poliovirus, human rhinovirus, and coxsackievirus has been shown to require the recruitment of a myriad of host cellular factors into the Golgi compartment. Among the key components is phosphatidylinositol 4-kinase III beta (PI4KIIIβ). PI4KIIIβ is a lipid kinase that resides mostly in the ER/Golgi and converts phosphatidylinositol to phosphatidylinositol-4-phosphate (PI4P), enriching the PI4P pool in the Golgi. Since PI4KIIIβ is a crucial host factor for many viruses, PI4KIIIβ is an attractive drug target with a potential for multiviral therapeutic indications. We solved the structure of a truncated form of PI4KIIIβ including the kinase domain at high resolution by X-ray crystallography. The structures for PI4KIIIβ were broadly similar to PI3K structures, but a unique helix near the ATP binding site, not predicted by homology modeling, was discovered. Leveraging of this structural knowledge accelerated the design of inhibitors highly selective for PI4KIIIβ over other lipid and protein kinases. Three scaffolds of ATP competitive reversible inhibitors were investigated for biochemical and antiviral potency, kinase selectivity, antiviral efficacy, and safety in vivo. All demonstrated a remarkable selectivity for PI4KIIIβ among over 300 protein/lipid kinases profiled biochemically including PI3Kα, β, γ, d, and PI4KIIIγ, in vitro antiviral activity against poliovirus, rhinovirus strains, and coxsackievirus, and in vivo activity against CVB4 induced pancreatitis. The medicinal chemistry lead optimization from initial hits to 7-day animal safety candidate compounds will be presented.
Benzhydrylpiperazine Compounds Inhibit Cholesterol-dependent Cellular Entry of Hepatitis C Virus
Ana M. Chamoun-Emanuelli, Zhilei Chen
Texas A&M University, College Station, USA

Hepatitis C virus (HCV) remains a serious global health problem that lacks an effective cure. Although the introduction of protease inhibitors to the current standard-of-care interferon/ribavirin therapy for HCV infection has improved sustained virological response of genotype 1-infected patients, these inhibitors exacerbate already problematic side effects. Thus, new HCV antivirals are urgently needed. Using a cell-protection screen previously developed in our laboratory, we evaluated 30,426 compounds for inhibitors of potentially any stage of the HCV life cycle and identified 49 new HCV inhibitors. The two most potent hits, hydroxyzine and chlorcyclizine, belong to the family of benzhydrylpiperazines and were found to inhibit the entry of cell culture-produced HCV with IC_{50} values of 19 nM and 2.3 nM, respectively, and therapeutic indices of >500 and >6500. Both compounds block HCV entry at a late stage of entry concomitant with viral fusion and their inhibitory activities are highly dependent on the cholesterol content of both the virion and host. Both compounds are currently used in the clinic for treating allergy-related disorders and the reported peak plasma level (160 nM) and estimated liver concentration (1.7 mM) of hydroxyzine in humans are much higher than the molecules’ anti-HCV IC_{90} in cell culture (64 nM). Further studies are therefore justified to evaluate the use of these molecules in an anti-HCV therapeutic regimen.

Identification of an Inhibitor of HIV-1 Vif-dependent Degradation of APOBEC3G
Erez Pery\textsuperscript{1,2}, Ann Sheehy\textsuperscript{3}, N. Miranda Nebane\textsuperscript{4}, Marie K. Mankowski\textsuperscript{5}, Lynn Rasmussen\textsuperscript{4}, E. Lucile White\textsuperscript{4}, Roger G. Ptak\textsuperscript{4}, Dana Gabuzda\textsuperscript{1,6}

\textsuperscript{1}Dept. Cancer Immunology and AIDS, Dana Farber Cancer Institute, Boston, USA, \textsuperscript{2}Dept. Pathology, Harvard Medical School, Boston, USA, \textsuperscript{3}Dept. Biology, College of the Holy Cross, Worcester, USA, \textsuperscript{4}High Throughput Screening Center, Southern Research Institute, Birmingham, USA, \textsuperscript{5}Dept. Infectious Disease Research, Southern Research Institute, Frederick, USA, \textsuperscript{6}Dept. Neurology (Microbiology), Harvard Medical School, Boston, USA

BACKGROUND: APOBEC3G (A3G) is a cellular cytidine deaminase that restricts HIV replication by inducing G-to-A hypermutation in viral DNA and by deamination-independent mechanisms. Vif overcomes this innate antiviral activity by binding to A3G and targeting it for proteasomal degradation, thereby inhibiting its incorporation into virions. The Vif-A3G interaction is essential for viral replication, making it a potential therapeutic target.

METHODS: A homogeneous TR-FRET assay was developed and optimized for high-throughput screening. Dose-response testing and counter screen assays were used to validate hits and eliminate false-positives. Confirmed hits were tested in cell-based assays to assess antiviral activity, demonstrate inhibition of Vif-dependent degradation of A3G, and further characterize effects on Vif and A3G.

RESULTS: Screening of 307,520 compounds identified 3,686 hits. Dose-response and counter screens confirmed 310 hits. Cell-based assays identified 3 compounds with antiviral activity that also attenuated Vif-dependent degradation of A3G. One compound, N.41, had antiviral activity in H9 cells (A3G+) but not SupT1 cells (A3G-), and dose-dependently reduced HIV-1 replication in PBMCs. N.41 increased A3G virion incorporation, attenuated single-round virus infectivity in a Vif-dependent manner, increased endogenous A3G levels, and inhibited Vif-A3G interaction in co-IP assays. These effects were specific to human A3G and not observed with African green monkey A3G. Preliminary SAR identified a hydroxyl moiety critical for N.41 activity and several N.41 analogs with improved activity.

CONCLUSIONS: N.41 attenuates HIV replication by inhibiting Vif-A3G interaction and increasing A3G innate antiviral activity. SAR studies suggest N.41 is a lead for further development as an antiviral.
A Single Mutation in the Chikungunya Virus RNA Polymerase Causes Resistance to Favipiravir (T-705)

Nidya Segura Guerrero¹, Leen Delang¹, Ali Tas², Gilles Querat³, Byron Martina⁴, Johan Neyts¹, Martijn van Hemert², Pieter Leyssen¹

¹University of Leuven, Leuven, Belgium, ²Leiden University Medical Center, Leiden, Netherlands, ³UMR_D 190, Aix-Marseille University, Marseille, ⁴Erasmus Medical Center, Rotterdam, Netherlands

T-705, also known as Favipiravir, is a small-molecule inhibitor that is currently in clinical development for the treatment of influenza virus infections. This molecule also inhibits the replication of a broad spectrum of other RNA viruses. In the host cell, T-705 is converted into its active metabolite, the ribonucleoside analogue T-705RTP. However, the precise molecular mechanism of action against influenza virus or any other virus remains to be elucidated. We here demonstrate that T-705 inhibits the replication of laboratory strains and clinical isolates of chikungunya virus (CHIKV) [EC₅₀ = 2-25 µM]. When given orally to CHIKV-infected AG129 mice in a pre- or post-exposure scenario, T-705 reduced virus-induced mortality by 85% and 65%, respectively. T-705 resistant CHIKV variants all acquired a K₂₉₁R mutation in motif F1 of the RNA-dependent RNA polymerase. Reverse-engineering of this mutation in an infectious clone of CHIKV corroborated the link between the mutant genotype and the compound-resistant phenotype. Interestingly, the lysine in motif F1 is also highly conserved in positive-stranded RNA viruses in general, again highlighting the importance of this amino acid. Although our observations do not exclude additional mechanisms of action against other viruses, this study lifts an important tip of the veil on the precise molecular mechanism of the antiviral activity of T-705 against (alpha)viruses and may help to design other potent broad-spectrum antivirals. Furthermore, this is the first report of resistance of any of the viruses that are susceptible to T-705. This research was funded by the European Union FP7 Program under SILVER grant agreement no. 260644.

Immune Modulators are Effective in Reducing Disease in a Mouse Model of Chikungunya

Ashley Dagley, Justin G. Julander
Institute for Antiviral Research, Utah State University, Logan, USA

Chikungunya virus (CHIKV) has recently emerged as a significant viral disease throughout the world. We have established a mouse model of pandemic Chikungunya (LR06) that demonstrates relevant disease, including arthralgia. As virus titers in various tissues decline as a result of host clearance, disease manifestations such as footpad swelling and splenomegaly increase, underscoring the importance of immunopathology in the development of disease. The purpose of this study was to characterize the effect of two immunomodulatory agents, methotrexate (MTX) and Actemra (ACT), an anti-IL-6 antibody, on CHIKV in our mouse model. These FDA-approved drugs were selected based on their use in the treatment of rheumatoid arthritis in people. The DBA/1J mouse strain was used and a 10⁴.₅ CCID₅₀ virus challenge was administered via footpad/hock injection under anesthesia. Daily subcutaneous treatment with MTX at 2 mg/kg/d for 7 days resulted in significant reduction of footpad swelling and splenomegaly. Similar results were observed after a single intraperitoneal treatment with ACT 4 hours after virus challenge at a dose of 40 mg/ml. Levels of IL-1α, MCP-1, MIP-1α, IFN-γ, and RANTES, which have previously been shown to be elevated during CHIKV infection, were significantly reduced in the hind limb and spleen of mice that were treated with MTX or ACT. There was also a reduction in IL-6 as compared to mean values of this cytokine in placebo-treated controls, although this reduction was not significant. Virus titer, however, was not reduced by treatment. Lower doses of 20 and 10 mg/ml of ACT were not effective. A combination of MTX and ACT did not improve disease beyond monotherapy treatment, although significant reduction in the aforementioned evaluation parameters was observed. Combination of MTX with 40 mg/ml of ACT resulted in a significant increase in virus titer possibly indicating inhibition of viral release. We have demonstrated efficacy of MTX and ACT in reducing disease in mice after CHIKV challenge. [Supported by HHSN272201000039I Task Order A21 from the Virology Branch, NIAID, NIH]
Targeting Membrane-bound Viral RNA Synthesis Reveals Potent Inhibition of Diverse Coronaviruses Including the Middle East Respiratory Syndrome Virus

Anna Lundin¹, Ronald Dijkman²,³, Tomas Bergstrom¹, Volker Thiel²,³,⁴, Edward Trybala¹

¹University of Gothenburg, Gothenburg, Sweden, ²Institute of Immunobiology, Kanontal Hospital, St Gallen, Switzerland, ³Federal Institute of Virology and Immunology, Berne, Switzerland, ⁴University of Berne, Berne, Switzerland

Coronaviruses (CoV) are enveloped, positive stranded RNA viruses that can cause severe respiratory illness in humans such as these caused by SARS-CoV and most recently by MERS-CoV. In spite of the fact that some CoV can be regarded as potential pandemic-causing viruses, no specific antiviral drug is currently available for treatment. Here we report on the further characterization of our anti-CoV compound K22, and propose a novel inhibitory mechanism for CoV infection. The modification of intracellular membranes and membrane-bound RNA synthesis is a characteristic feature during CoV replication and treatment with K22 resulted in inhibition of double membrane vesicles (DMV) formation and near complete inhibition of RNA synthesis. K22-resistant viruses displayed amino acid substitutions in non-structural protein 6 (nsp6), a membrane-spanning integral component of the viral replication complex involved in DMV formation. Viruses with mutations in nsp6 displayed a reduced specific infectivity as well as a reduction in DMV formation but with maintained RNA synthesis compared to wild-type virus implying that viral variants comprising mutated nsp6 can produce infectious particles with reduced fitness. The reduced fitness of nsp6 mutants cannot be ascribed to inadequate package of viral RNA. Furthermore, K22 was shown to exhibit antiviral activity against a range of human and animal coronaviruses including MERS-CoV which was also studied in primary human airway epithelial cells. In summary, we show that the membrane-bound coronavirus RNA synthesis is a drug-vulnerable event of the viral life cycle and a promising target for antiviral intervention.

Mechanism of Inhibition for BMS-791325, a Non-nucleoside Inhibitor of Hepatitis C Virus NS5B Polymerase in Phase 3 Clinical Studies

Karen Rigat, Hao Lu, Ying-Kai Wang, John Kadow, Min Gao, Lynn Abell, Julie Lemm, Susan Roberts

Bristol-Myers Squibb Co., Wallingford, USA

BMS-791325 is a novel direct-acting antiviral (DAA) specifically targeting hepatitis C virus (HCV) NS5B, an RNA-dependent RNA polymerase. Co-crystal structures and resistance selection in HCV replicon cells demonstrated that this non-nucleoside inhibitor interacts with a site in the thumb domain of the polymerase (thumb site I). Robust clearance of HCV was observed in Phase 2 clinical studies in infected patients treated with BMS-791325 in combination with other anti-HCV agents. The preclinical profile of BMS-791325, including potent inhibition of multiple genotypes in enzyme and replicon assays (GT-1 IC₅₀ and EC₅₀ values of 2-10 nM), selection of significant resistance at a single substitution site, and a robust pharmacokinetic profile in animal models, anticipated the strong antiviral effect observed in patients. Biochemical and biophysical studies revealed an inhibition mechanism likely to play a role in the clinical effectiveness of the inhibitor. BMS-791325 is a time-dependent, non-competitive inhibitor of polymerase initiation. Binding studies with wild type (WT) NS5B and genetic variants (L30S and P495L) exposed a two-step, slow-binding mechanism. Details of the binding mechanism differed for WT and mutant polymerases. The rate of initial complex formation and dissociation is 7-10 times faster for a fingers domain variant (L30S) compared to WT however, the affinity to form the final complex is not significantly different. A clinically relevant resistance variant in the thumb domain (P495L) has a significantly different profile. The rate of initial complex formation and dissociation is similar for P495L and WT, but the kinetics of the second step are significantly faster, showing that this variant impacts the final tight complex. The resulting shortened residence time translates into the observed decrease in inhibitor potency. The impact of the genetic variants on the behavior of BMS-791325 provides direct experimental evidence for a dynamic interaction between the fingers and thumb.
**93 Characterization of MBX2329 and MBX2546- Unique Small Molecule Inhibitors of Influenza A Virus**
Arnab Basu¹, Gloria Komazin-Meredith¹, Donald T. Moir¹, Dale L. Barnard², Lijun Rong³, Terry L. Bowlin¹
¹Microbiotix Inc, Worcester, USA, ²Utah State University, Logan, USA, ³ University of Illinois at Chicago, CHICAGO, USA

The influenza virus envelope glycoprotein hemagglutinin (HA) is a potential target for antiviral drugs because of its key roles in the initial stages of infection: receptor binding and the fusion of virus and cell membranes. Recently, we reported two influenza virus inhibitors (Basu et al, J. Virol accepted) MBX2329 and MBX2546, with aminoalkyl phenol ether and sulfonamide scaffolds respectively, that specifically inhibit HA-mediated viral entry in H1 and H5 strains. The two compounds are (a) potent (IC₅₀ = 0.3–5.9 µM), and (b) selective (CC₅₀ >100 µM) with selectivity index (SI) values >20-200 for different influenza strains. Studies presented herein show that the inhibition is mediated through specific interaction with the HA protein. In an attempt to define the binding pocket within the HA molecule, a number of drug-resistant viruses have been isolated and characterized. Sequence analyses of the HA gene of these drug-resistant viruses mapped amino acid changes responsible for drug resistance to a region located near the amino terminus of HA2. Additional studies, that includes (i) scanning alanine mutation in the stem region (ii) competition assays with MAb C179, and (iii) preliminary modeling studies further supports the hypothesis. Together, the results of these studies indicate that MBX2329 and MBX2546 fall within a class of inhibitor which interacts with HA to stabilize it against the low pH transition to its fusogenic state and consequently inhibit HA-mediated membrane fusion during influenza virus infection.

**94 The Selectivity of Cidofovir for HPV-Positive Cells is Based on the Differential Response to DNA Damage of Normal Cells and Cancer Cells**
Graciela Andrei, Tilm De Schuttter, Dimitrios Topalis, Lieve Naesens, Robert Snoeck
Rega Institute for Medical Research, Leuven, Belgium

PURPOSE. Cidofovir (CDV) has proven effective in treatment of HPV hyperplasias. CDV antiproliferative effects were ascribed to apoptosis induction, accumulation of cells in S-phase, and increase in p53, pRb and p21 protein expression. We aimed to characterize the molecular mechanisms for the selectivity and antiproliferative activity of CDV against HPV-transformed cells by whole human genome microarray analysis.

MATERIALS AND METHODS. Gene expression changes following CDV treatment of different cell types [including, two HPV⁺ cervical carcinoma cell lines (SiHa and HeLa), an HPV⁻ immortalized keratinocyte cell line (HaCaT), and primary human keratinocytes (PHKs)] were evaluated. Metabolic studies and drug incorporation into genomic DNA were analyzed in the four cell types using radiolabel compound.

RESULTS. A higher rate of drug incorporation in immortalized cells compared to normal keratinocytes was observed. Distinct and specific drug effects in the different cell types were shown by gene expression profiling. Although an effect on inflammatory response was seen in all cell types, different pathways were identified in normal keratinocytes versus immortalized cells. Notably, Rho GTPase pathways, LXR/RXR pathways, and acute phase response signaling were exclusively activated in immortalized cells. CDV-exposed normal keratinocytes activated cell cycle regulation upon DNA damage signaling to allow DNA repair via homologous recombination, resulting in genomic stability and survival. In HaCaT cells, CDV induced cell cycle arrest but DNA repair was not activated while apoptosis of tumor cell lines was activated following in CDV-treated HPV⁺ cells.

CONCLUSIONS. CDV selectivity is based on the failure of HPV⁺ cells to respond to DNA damage rather than on a direct anti-HPV effect. Since cell cycle control is deregulated by the viral oncoproteins E6 and E7 in HPV⁺ cells, these cells are more susceptible to DNA damage than normal keratinocytes. Our findings underline the therapeutic potential of CDV for HPV-associated malignancies as well as other neoplasias.
Inhibition of Dengue and Chikungunya Virus Infection by RIG-I-mediated IFN-independent Stimulation of the Innate Immune Response

David Olagnier¹, Florine Scholte², Cindy Chiang², Irina Albulescu¹, Rongtuan Lin¹, Eric Snijder², John Hiscott¹, Martijn van Hemert²

¹Vaccine & Gene Therapy Institute of Florida, Port St. Lucie, USA, ²Leiden University Medical Center, Leiden, Netherlands, ³McGill University, Montreal, Canada

Dengue virus (DENV) and chikungunya virus (CHIKV) are mosquito-borne viruses that can cause severe arthralgia and affected millions of people in the past decade. Unlike CHIKV, DENV causes over 10,000 deaths annually, which are associated with antibody-dependent enhanced severe infections. The geographic expansion and increased incidence of DENV infections as well as the re-emergence of CHIKV in an epidemic form that recently even spread to the Caribbean, highlight the relevance of these human pathogens. The current lack of registered vaccines and effective antivirals stresses the importance of investigating new strategies to combat these pathogens. Ideally, such strategies should target both viruses, as they are endemic in many of the same regions, in which the capacity to perform (differential) diagnosis is often limited. The cytosolic sensor RIG-I plays an important role in the innate immune response to RNA virus infection. We have evaluated the inhibitory effect of a RIG-I agonist, an optimized 5' triphosphorylated RNA (5’pppRNA) molecule, on DENV and CHIKV replication. Treatment of cells with low, non-cytotoxic doses of 5’pppRNA led to RIG-I stimulation and generated a robust antiviral response against these two viruses. Strikingly, even 5’pppRNA treatment after DENV or CHIKV challenge provided protection against infection. In primary human monocytes and monocyte-derived dendritic cells, 5’pppRNA blocked both primary infection and antibody-enhanced DENV infection. The 5’pppRNA-induced protective response against DENV and CHIKV was dependent on an intact RIG-I/MAVS/TBK1/IRF3 axis, but was largely independent of the type I IFN response. Altogether, this in vitro analysis of the antiviral efficacy of 5’pppRNA highlights the therapeutic potential of RIG-I agonists. This work was supported by VGTI Florida, the Canadian Institutes of Health (grant nr. CCI-249187), and the EU-FP7 projects SILVER (grant nr. 260644) and EUVIRNA (grant nr. 264286).

New Treatment Protocol Targeting Dengue Virus and Dengue Induced Cytokine Excess/Storm and Assured Prophylaxis with Functionally Metamorphosed Drugs Already in Use.

M. Chandramohan¹, D. Sivakumar¹, S.C. Vivekananthan¹, M. Kannan¹, P. Selvam²

¹ Kamarajar liver hospital and Research Centre, Madurai, India, ²Nova College of Pharma. Edu and Research, Jupudi, India

Medical fraternity all over the world lamenting that there is no specific drug for Dengue virus fever or vaccine and opting for symptomatic treatment and trying in futile to control the vector mosquitoes Aedeses. It is imperative to search and formulate specific therapy protocol and assured prophylaxis in this new millenium. Our search in world literature yielded Dengue ANTIVIRALS: a) Chloroquine[CQ] two modes of antiviral actions cell entry blocker and NS3 blockers(b)S-Adenosyl LMethionine [SAMe] binds NS5 Methyl Transferase(c)Doxycycline [D.Cy] blocking viral entry and replication at NS2B & NS3 proteases and all the three of them had shown to have functional metamorphosis as antidengue viral activity agents. We have added the broad spectrum antiviral Ribavirin[RV]. ANTICYTOKINES:CQ and D.Cy got selected again both block TNFalpha,IL1B,IL-6and had metamorphosed as anti-cytokine along with Zileuton[ZN] blocking cysteinyl leukotriene and leukotrieneB4 and Monteleukast [ML] blocking cysteinyl receptor.
MATERIAL AND METHOD: Recruited 7 male 4 female patients age ranged between 16 and 36, who had fever, with conjunctival injection, body pain and retrobulbar pain who were positive for NS1 antigen by rapid card test and the remaining sera tested for NS1 antigen, antidengue antibodies IgM and IgG by ELISA assays proved positive denoting secondary dengue infection. All the six drugs given from the day one of the diagnosis in the following oral dose
1. CQ 300mg o.d 2. SAMe 400mg tds 3) RV 200mg qid 4) D.Cy100mg b.d 5) ZN600mg b.d 6) MT10mg o.d Fever and other symptomatologies abated in 5 to 14 days while dengue IgM and IgG ELISA repeated on every 4 to 6 days and noticed that Dengue AntiIgM and IgG rendered negative in 16 to 30 days by ELISA while these antibodies usually persist in the blood for 90 days and lifelong respectively. These results denote that prompt two prong attack on dengue virus and the cytokine of the illness with multimodal action a formidable combi-pack might have drastically restricted the DV load and yielded minimal antibodies production which were cleared very early and interpreted as “RADICAL CURE”

100 The Dengue Virus NS1 Protein Modulates Innate Immune Signaling Early During Infection
Farah Alayli, Frank Scholle
NC State University, Raleigh, USA

Dengue Virus (DenV) is a positive sense ssRNA virus belonging to the Flaviviridae family. DenV is transmitted to humans by mosquito vectors and typically causes self-limiting dengue fever (DF). Severe cases can progress to dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), both life-threatening conditions, which are thought to be caused by high viremia. Non-structural protein 1 (NS1) is a secreted glycoprotein that is expressed by all flaviviruses and is necessary for viral replication. NS1 West Nile Virus (WNV), a closely related virus, has been shown to inhibit TLR3 signaling and disrupt complement activation. DenV NS1 is found in the blood of infected patients and is thus used as a diagnostic marker, however its immunomodulatory functions remain unknown. Based on our findings with the WNV analog, we hypothesize that DenV NS1 inhibits early immune activation during infection. This would serve as an immune evasion strategy potentially leading to more virus replication and spread. Initially we demonstrate that DenV infection of HeLa and mo-DCs results in inhibition of TLR3 signaling. DenV NS1 protein was purified and HeLa cells that stably express the protein (HeLaNS1) were established. Upon TLR3 stimulation of either NS1 pretreated or NS1 expressing HeLa cells, a reduction in IL-6 production compared to the control was detected. Furthermore, upon DenV infection of HeLaNS1 or control cells, HeLaNS1 cells secreted less IL-6, without an effect on IFNβ cytokine levels or DenV production from infected cells. In order to put these findings in the context of a primary and early target of dengue infection, the effect of NS1 on mo-DCs, was investigated. Mo-DCs take up soluble NS1, and upon infection or LPS stimulation, are not able to up-regulate maturation markers compared to the control. Collectively, our data suggests that NS1 transiently modulates innate immune signaling very early during dengue infection.

101 Antiviral NanoNase-TM
Jacob G. Appelbaum, Rudolf I. Salganik
AVIRID, INC., Gainesville, USA

Currently there is practically no broad-spectrum antiviral therapeutics most existing antiviral drugs are inhibitors of a virus-specific target, resulting in drug resistant viral strains when virus mutates the target and high toxicity particularly when applied as drug cocktails to delay viral resistance. Enormous viral diversity makes it abundantly clear – virus-specific drugs are not the answer to serious threat posed by viral pathogens many still unidentified. We have developed a novel broad spectrum antiviral platform termed NanoNase™ for selective destruction of viral nucleic acids replicating or dormant inside virus-infected cells. NanoNase™ is modular multi-shell nanocomplex consisting of core module comprised of nanocarrier bearing non-specific endonucleases surrounded by a shell formed by ligands binding highly-conserved sequence of targeted viral nucleic acid, RNA or DNA, and further surrounded by a shell armed with ligands facilitating entry into cells infected by targeted virus [Fig 1]. The original concept of the antiviral NanoNase™ has been disclosed by the authors in 2004 patent application [US20090047272]. Most recently remarkable antiviral efficacy of anti-HCV NanoNase™ has been independently confirmed using Au-nanoparticle complex with RNase A and DNA-oligonucleotides complimentary to highly conserved sequence of HCV RNA: 0.3pM of the complex reduced viral load in mice by 99.6% without any measurable toxicity [Wang et al, 2012]. The current objective is to design biodegradable non-specific core module of
NanoNase™ that can be pre-manufactured and directed against virtually any viral or cellular nucleic acid by simple assembly of nucleic acid-targeting and cell-entry enabling components. NanoNase™ may be further modified to enable topical or oral delivery.

**102 Unique Features Identify CCR5-Using Macrophage-Tropism as a Rare Phenotype of HIV-1 Distinguishable from CCR5-Using T Cell-Tropism**

Kathryn T. Arrildt¹, Sarah B. Joseph¹, Celia C. LeBranche², Ean Spielvogel¹, Zaki Dard¹, David C. Monetefiori², Ron I. Swanstrom¹

¹University of North Carolina, Chapel Hill, USA, ²Duke University, Durham, USA

Macrophages have been implicated as target cells in many stages of HIV-1 infection: transmission/acute, chronic, late, and reservoir. However, identification of macrophage-tropic (M-tropic) viruses has hitherto been difficult, in part because macrophages vary widely in susceptibility to infection. Quantification of cell-surface CD4 revealed that macrophage have densities 25-fold lower than T cells, a potentially significant obstacle for infection, and that CD4 density was strongly correlated with susceptibility to infection. Using rigorously defined M-tropic viruses (produced from a long-lived cell in vivo and able to efficiently infect monocyte-derived macrophages) to infect Affinofile cells (a novel cell line with titratable CD4 density) revealed that M-tropic viruses have a much lower entry requirement for CD4 density than to patient-matched T cell-tropic (T-tropic) viruses. M-tropic viruses are more sensitive to soluble CD4 and some antibodies targeting the CD4 binding site. Preliminary studies of the genetic determinants indicate changes in residues that interact with CD4. However, sensitivity to antibodies targeting the coreceptor (CCR5) binding site was indistinguishable between M- and T-tropic viruses. Similarly, titration of maraviroc (a CCR5-blocking drug) showed no difference, implying that differences in entry are primarily due to CD4 usage. No difference was detected in Env conformation by sensitivity to polyclonal sera or heat inactivation. Screening for CD4 usage from a large number of acutely and chronically infected subjects, we were unable to detect any M-tropic viruses, indicating that it is a very rare phenotype and unlikely to play a role in transmission/acute or chronic infection. So far we have detected M-tropic viruses only in the cerebrospinal fluid of subjects late in disease with neurocognitive defects. Identification of the genetic determinants of M-tropism would enable screening using next generation sequencing and we continue to explore the phenotypic consequences of M-tropism, which may reveal targets to aid prevention of HIV-associated neurocognitive complications.

**103 Efficacy of the Beraprost Isomer GP1681 for Treating Influenza Virus A H5N1 Infections in Mice**

Dale Barnard¹, Jiing-Huey Lin², William Guilford², Daryl Faulds²

¹Utah State University, Logan, USA, ²Gemmus Pharma, San Francisco, USA

Beraprost sodium (BPS), a mixture of 4 isomers, significantly enhanced the survival of influenza A H5N1 virus infected mice (Abst. 119, ICAR 2013). To reduce potential unwanted side effects from inactive stereoisomers, BPS was separated into single isomers for testing in influenza A H5N1 virus infection mouse models. For mice treated with isomer A (GP1681) at 0.8 mg/kg/d (bid X 10, i.p.) 40% of mice survived (P< 0.0001) versus 30% for BPS-treated mice (1.6 mg/kg/d, bid X 10, i.p.; p< 0.001). The mean day of death for GP1681-treated mice was 11 days versus 7.7 days for placebo-treated mice. Other isomers were not active. To evaluate possible drug-drug interactions with standard influenza virus therapiies, GP1681 was used in combination with oseltamivir. ALL mice survived when treated with GP1681 (0.8 mg/kg/d, bid X 10, i.p.) and low dose oseltamivir (1.0 mg/kg/d, bid X 5, p.o. P< 0.0001) with 60% (p< 0.001) and 80%
Abstracts

Innate Immune Agonists Demonstrate Pre-clinical Efficacy and Tolerability Representing a Novel Class of Broad Spectrum Antivirals

Kristin Bedard¹, Ikenna Madu¹, Shari Kaiser¹, Myra Wang¹, Michael Gale, Jr², Shawn Iadonato¹
¹KINETA, Inc, Seattle, USA, ²University of Washington, Seattle, USA

We have identified a novel class of small molecule antiviral drugs that are effective against diverse RNA and DNA viral pathogens through host directed antiviral mechanisms. Traditional antiviral drug design has failed to identify broad spectrum drugs, while direct acting antivirals available for select pathogens are plagued with the development of resistant strains that render them ineffective. Drug development focused on host directed molecules has been of great interest over the last decade; however, there are few examples of potential drugs in pre-clinical and early clinical development. We have identified a novel class of small molecule drugs that trigger a natural immune response by targeting the innate immune transcription factor, IRF-3, a critical first responder that is essential for suppressing viral replication and clearing infection. We report on two unique scaffolds that stimulate IRF-3 activity and cause a potent inhibition of viral pathogens including RNA and DNA viruses. One compound class includes isoflavone drugs that are being developed as broad spectrum therapeutics for respiratory pathogens including Influenza viruses, paramyxoviruses, and coronaviruses. The optimized isoflavone leads are orally bioavailable, well tolerated in vivo and show antiviral efficacy (reduction in virus titers and enhanced survival) in pre-clinical infection models for Flu and MHV (a SARS-like model in rodents). A distinct set of compounds that includes benzothiazole drugs are also being considered for pre-clinical development due to their oral bioavailability, in vivo tolerability and broad spectrum efficacy against respiratory viruses, Flaviviruses (including Dengue and West Nile virus) and clinically important herpesviruses (including CMV). Pre-clinical data on this novel drug target will be discussed and represents the promise of host directed innate immune stimulators for future clinical development. This is the first report of innate immune agonist lead selection and represents a favorable new target for broad spectrum antiviral drug development.

Mode of Action of GP1681 as a Therapeutic for Influenza A Infections

Daryl Faulds¹, Dale Barnard², William Guilford¹
¹Gemmus Pharma Inc, San Francisco, USA, ²Utah State University, Logan, USA

Influenza A virus (IAV) infects the host’s respiratory tract epithelium and thereby induces a vigorous immune/inflammatory response. It is hypothesized that modulation of the host response and improved resolution of inflammation may be a way for treating IAV infections. The pathogenesis and immune response associated with IAV infection can be studied in the mouse model. We have previously demonstrated that Beraprost Sodium (BPS, GP1001) significantly improves survival of BALB/c mice infected with IAV H5N1 compared to placebo-treated mice. We found that GP1001 significantly reduces both inflammatory cytokine levels and the acute mononuclear cellular infiltrate associated with immune pathology. GP1001 is a mixture of four stereoisomers. In order to reduce the chance of unwanted side effects arising from unneeded and/or inactive stereoisomers the four stereoisomers which comprise GP1001 (Isomers A-D) were isolated and individually evaluated in the H5N1 mouse model. There was no significant survival difference between placebo-treated and GP1001 Isomers B, C and D-treated groups. The survival endpoint showed a significant improvement for GP1001 Isomer A-treated, and GP1001-treated mice compared to placebo-treated mice. Cytokines were
evaluated by multiplex analyses of lung homogenates. The concentration of pro-inflammatory cytokines IFN-γ, IL-6, IL-10 and IL-12 and chemokines CCL2 (MCP-1) and CCL5 (RANTES) were increased significantly (P < 0.05) in lung homogenates of virus-infected, placebo-treated mice compared to uninfected mice at day 6 post-infection. Although treatment with Isomers B, C, and D did not significantly reduce the level of any cytokine compared to virus-infected, placebo-treated mice, treatment with Isomer A (GP1681) and GP1001 significantly reduced (P < 0.05) the concentration of IFN-γ (60%), and CCL2 (55%). In addition, treatment with Isomer A (GP1681) reduced the concentration of cytokines IL-6 (55%) and IL-10 (75%) and chemokine CCL5 (52%) in the lung homogenates compared to virus-infected, placebo-treated mice. Since the cytokine profile of hospitalized human infections is somewhat similar to the H5N1-infected mouse, these results suggest that GP1681 may be a useful therapeutic for treating influenza infections.

106 Nanoemulsion-Adjuvanted Vaccines Induce Robust Protection Against Genital HSV-2 Infection in a Guinea Pig Model

R. Cardin1, F. Bravo1, T. Hamouda2, V. Bitko2, C-A. Malinczak2, J. Sun2, A. Fattom2, D. Bernstein1
1Cincinnati Children's Hospital Medical Center, Cincinnati, USA, 2NanoBio Corp., Ann Arbor, USA

A vaccine against genital herpes is a health priority but recent vaccines have failed to protect against HSV-2 in humans. The novel nanoemulsion (NE80) induces both Th1/Th2 immune responses and mucosal immunity. Therefore, NE80-adjuvanted whole HSV-2 or subunit gD2 vaccines were evaluated in the genital HSV-2 guinea pig model. Nanoemulsion is an oil-in-water emulsion comprised of oil, cationic surfactant (cetylpyridinium chloride), non-ionic surfactant (Tween-80), and an organic solvent. Antigen incorporation into nanodroplets can be delivered as vaccines by intramuscular (IM) or intranasal (IN) routes. In this study, we compared the following vaccines: 1) HSV-2/NE80 IM, 2) HSV-2/NE80 IN, 3) Inactivated HSV-2 only IN, 4) gD2/NE80 IM, 5) gD2/NE80 IN 6) gD2/MPL/Alum IM, and 7) No vaccine. Female guinea pigs were vaccinated at 0, 3, 6, and/or 9 weeks (n = 12/group). Sera and vaginal wash were analyzed for anti-HSV-2, gD2 and gB2 antibodies and for HSV-2 neutralizing antibodies. Animals were challenged with 1 x 10⁶ pfu HSV-2 and followed for acute disease and recurrences disease. Following HSV-2 challenge, the HSV-2/NE80 IN, gD2/NE80 IM, gD2/NE80 IN vaccines, and control gD2/MPL/Alum vaccine significantly reduced the severity of acute disease (p < 0.0002) as well as recurrent disease (p < 0.03). Vaginal virus titers in the gD2 vaccine groups were significantly reduced at 2 and 6 days post challenge compared to the No vaccine placebo group. The HSV-2/NE80 IM vaccine elicited high levels of anti-HSV-2 and gD2 antibodies in both sera and vaginal washes, whereas anti-gD2 antibody levels were significantly lower (p < 0.0001) compared to the gD2/MPL/Alum vaccine. The gD2/NE80 IN vaccine elicited lower serum anti-gD2 antibodies and neutralizing antibodies compared to the gD2/MPL/Alum vaccine, yet showed comparable protection. Thus, protection was achieved with gD2 when administered with NE80 by either IM or IN route. These data suggest that the administration of gD2 by either the IM or IN route or a whole virus vaccine by the IM route with NE80 is effective and warrants further optimization and evaluation.

107 Exploring the Mechanisms of Action for Respiratory Syncytial Virus Inhibitors

Sreerupa Challa, Choi Lai Tiong-Yip, Qin Yu
Infection Innovative Medicines Unit, AstraZeneca R&D Boston, Waltham, USA

Human Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract illness in young children and elderly individuals characterized by pneumonia and bronchiolitis. Currently there is no specific treatment for RSV infection except for anti-RSV antibody (Synagis) that is prescribed only for high risk populations. There is an urgent need for development of safe and effective drugs to treat this unmet medical need. In this context, small molecule compounds that potentially target different components of the viral replication machinery have been developed. In an attempt to qualitatively and quantitatively determine the effect of these inhibitors on viral replication, we performed RSV infection and subgenomic replicon assays, RT-qPCR, confocal microscopy, and resistance analysis in the presence of selective of RSV inhibitors. Varied effects on RSV RNA replication, Localization of inclusion bodies, and cell-line dependent potency were observed. The results have revealed the strength and weakness for each class of compounds, providing important scientific knowledge to guide our strategy to discover and develop novel inhibitors for RSV therapy.
**108 Study on Inhibitors of the Herpes Simplex Virus Type 1 Alkaline Nuclease**

*Tian Chen*, Lei Zhang, Hongyu Chen, Kang Cao, Jin Hu, Qu Pan  
*Chengdu Medical College, Chengdu, China*

**AIM:** In order to find an effective antiviral drug against the herpes simplex virus type 1 (HSV-1) and understand its mechanism, we have screened alkaline endonuclease (AN)-dependent inhibitors.

**Methods:** We have constructed a plasmid, pET28-UL12, with an insertion of a DNA fragment containing the UL12 gene of HSV-1 SM44, which is 99.2% homologous to the UL12 (Gene ID: 2703382 from the GenBank) gene. After transformation in Escherichia coli, only kanamycin-resistant clones were selected. Expression of recombinant proteins in Escherichia coli restored the natural activity of the protein.

**RESULTS:** The anti-AN activities detected in several drugs extracted from herbs were confirmed by different degrees of inhibition on the enzymatic activities, similar to the research in Vero cells that exhibited different levels of inhibiting viral infection by these drugs. The result showed that most of these drugs can dramatically interfere with HSV-1 infection on the Vero cells, but only Baicalin can significantly inhibit the rAN and stop HSV-1 replication. Docking results showed that Baicalin had a strong interaction with critical amino acid residues of AE (identity 21.12%, similar residues 41.35%, compared with rAN by DNAMAN 6.0, data not shown) which was expressed by Epstein–Barr Virus.

**CONCLUSION:** This DNA degradation assay is a useful tool to rapidly screen the inhibitors of HSV-1 at the molecular level, also representing a potential target for novel antiviral therapies. Corresponding author: Tian Chen, Email: chentianchina@126.com. This work was financially supported by the National Natural Science Foundation of China (no. 81173637).

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**109 Inhibition of Hsp90 by Small Molecules Cures Human Herpesvirus 8 Persistence**

*Wuguo Chen*¹, Sang-Hoon Sin², Kwun Wah Wen³, Blossom Damania⁴, Dirk Dittmer⁵  
¹University of North Carolina, Chapel Hill, USA, ²University of North Carolina, Chapel Hill, USA, ³University of North Carolina, Chapel Hill, USA, ⁴University of North Carolina, Chapel Hill, USA, ⁵University of North Carolina, Chapel Hill, USA

Heat-shock protein 90 (Hsp90) inhibitors exhibit activity against human cancers. We evaluated a series of new, oral bioavailable, chemically diverse Hsp90 inhibitors (PU-H71, AUY922, BIIB021, NVP-BEP800) against Kaposi sarcoma (KS). All Hsp90 inhibitors exhibited nanomolar EC(50) in culture and AUY922 reduced tumor burden in a xenograft model of KS. KS is associated with KS-associated herpesvirus (KSHV). We identified the viral latency associated nuclear antigen (LANA) as a novel client protein of Hsp90 and demonstrate that the Hsp90 inhibitors diminish the level of LANA through proteasomal degradation. These Hsp90 inhibitors also downregulated EphA2 and ephrin-B2 protein levels. LANA is essential for viral maintenance and EphA2 has recently been shown to facilitate KSHV infection which in turn feeds latent persistence. Further, both molecules are required for KS tumor formation and both were downregulated in response to Hsp90 inhibitors. This provides a rationale for clinical testing of Hsp90 inhibitors in KSHV-associated cancers and in the eradication of latent KSHV reservoirs.
110 Development of an HIV-infected, Humanized Mouse Model with Antiviral Pharmacokinetic/Pharmacodynamic Capabilities

Milloni Chhabra, Jerry Jeffrey, Sonia Miranda, Angela Mote, Barbara Denton, Paula Gardner, Joe Watson, My-Nga Nguyen
GlaxoSmithKline, RTP, USA

We have developed a robust humanized mouse (hu-mouse) model capable of sustaining a systemic HIV infection, with the ability to measure pharmacokinetic/pharmacodynamic (PK/PD) properties to provide a relevant in vivo model for HIV research. To generate the humanized mice, four week old NOG mice were exposed to a sub-lethal dose of irradiation, and transplanted with CD34+ stem cells from cord blood. Eight weeks post-transplant, mice were tested for human CD45+ cells. From 136 mice, all animals were in the range of 22-96% hCD45 in the blood. To demonstrate susceptibility of the model to HIV infection, four strains of HIV were used to inoculate the hu-mouse. Virus was detectable in the plasma as early as one week post-infection, in the range of 10^3 to 10^6 RNA copies/mL. For BaL (R5-tropic), viral load (VL) steadily increased over time. For ASM54 (dual-tropic), VL was detectable at one week post-infection, and consequently undetectable. For CC1/85 (R5-tropic), two out of three mice had detectable and increasing VL over time, one mouse remained VL negative. Finally, for ASM57 (R5-tropic), the VL from week one post-infection remained steady. Viral kinetics differed between the strains of HIV however, there was no correlation with virus tropism. The BaL-infected mice were also subjected to PK studies using a protease inhibitor (GW640385X) in a long-acting parenteral (LAP) formulation via intramuscular injection. A group of four infected mice were dosed once every four days, with six doses over 24 days. The plasma exposure was consistently above target concentration for an antiviral response. Plasma VL in all four mice decreased an average of 1.4 log copies RNA/mL from peak VL (range = 1.0 and 2.2 log), showing a consistent PK/PD phenotype. The VL decline associated with GW640385X is a proof-of-concept to use the HIV-infected, humanized mouse model for antiviral PK/PD characterization. This model provides a valuable in vivo system for measuring tissue-specific exposure of compounds (ex: blood, thymus, spleen, bone marrow, liver, gut, CNS) and correlation with tissue-specific reservoirs of virus, which currently cannot be addressed with in vitro systems.

111 Formulation of cidofovir Improves the Anti-papillomaviral Activity of Topical Treatments in the CRPV/rabbit Model.

Neil D. Christensen, Nancy M. Cladel, Jiafen Hu, Balogh K. Karla
Penn State University, College of Medicine, Hershey, USA

Current topical treatments for papillomas use ablative, cytotoxic and immunomodulating strategies and reagents. However, the effectiveness of topical treatments using different formulations has not been examined in preclinical models or clinical trials. The purpose of this study was to determine whether formulation of the small molecule acyclic nucleoside, cidofovir, could lead to improved therapeutic endpoints following topical treatment of papillomas using the cottontail rabbit papillomavirus (CRPV)/rabbit model. Different formulations with a set dose of 1% cidofovir were tested to establish comparative data. The formulations compared included cidofovir in (a) saline, (b) 10% DMSO, (c) 1% carbomer 940 and (d) 50:50 cremophor emulsion. Each rabbit was infected at 4 back sites with wild-type CRPV (wtCRPV) and a mutant CRPV (mE8-CRPV) in which the E8 gene product was eliminated via an ATG mutation. The latter mutant grew papillomas that were substantially smaller than wtCRPV lesions. The results demonstrated that anti-papilloma treatments with topical cidofovir was greatly enhanced when formulated versus unformulated. The most effective treatments were found using cidofovir formulated in cremophor in which complete cures of all treated sites was observed. Carbomer 940 formulated cidofovir showed strong but variable effects leading to cures on some rabbits, but not others. No cures were obtained with cidofovir in saline or in DMSO. Further studies indicated that cremophor formulations of cidofovir led to complete cures of papillomas at dilutions less than 0.3% cidofovir. These and previous studies demonstrated that unformulated cidofovir under the same treatment regime was curative at 2% but not at 1%, demonstrating that much less compound can be used when properly formulated.
A Mouse Papillomavirus Model to Study Anti-viral Responses to Cutaneous and Anogenital Mucosal Infections.
Neil D. Christensen, Nancy M. Cladel, Lynn R. Budgeon, Karla K. Balogh
Penn State University, College of Medicine, Hershey, USA

Papillomaviruses are species-restricted pathogens that cause epithelial lesions of cutaneous and mucosal tissues. HPVs continue to cause significant morbidity and mortality and are associated with various cancers of the anogenital tract, head and neck, and some skin sites. Current preclinical models to study therapeutic responses of various antivirals, vaccines and other therapeutics are restricted to canine, bovine and rabbit papillomavirus models. None of these models include PV infections of genital mucosa. We have recently developed a mouse papillomavirus infection model based on the discovery of a mouse PV infection on the muzzle of athymic mice (Vet Pathol 48, 500, 2011). The virus was classified as a cutaneous-tropic virus. We and others have been able to demonstrate that other cutaneous sites are susceptible to mouse PV infections, especially the mouse tail. Our more recent studies have now demonstrated that the mouse PV can also directly infect mucosal tissues of the female genital and anal tracts. Extensive PV infection was observed in female mouse vaginal and anal tissues as determined by in situ hybridization to detect viral DNA and capsid-antigen staining to detect virions. Mild dysplasia of the genital epithelium was observed with some indications of increased pathology trending towards VIN2. This mouse PV infection model represents a novel preclinical model to test antiviral compounds for control of anogenital mucosal PV infections. There will be opportunities to formulate and test antiviral compounds that require in situ delivery into the female genital tract to better assess efficacy of compounds to control HPV genital infections.

A Small Molecule Inhibitor of Virion Attachment to Heparan Sulfate- or Sialic Acid-Containing Glycans
Che C. Colpitts, Luis M. Schang
Li Ka Shing Institute of Virology, Edmonton, Canada

Primary attachment to cellular glycan moieties is a critical step during entry of most human viruses. Some viruses, such as herpes simplex virus type 1 (HSV-1) and hepatitis C virus (HCV), bind to heparan sulfate moieties in cellular glycosaminoglycans, whereas viruses such as influenza A (IAV) bind to terminal sialic acid moieties in cellular sialoglycans. Both types of primary attachment involve low-affinity interactions between basic binding pockets in virion glycoproteins and negatively charged modified saccharides. Receptor mimetics that interfere with these interactions are active against viruses that bind to either heparan sulfate or sialic acid. However, no single molecule has been identified that inhibits the attachment of viruses in both groups. Consequently, no truly broad-spectrum clinical antivirals that target attachment exist. Epigallocatechin gallate (EGCG), a green tea catechin, inhibits the infectivity of a diverse group of enveloped and nonenveloped human viruses. We sought to identify the basis for its broad-spectrum activity. Here, we show that EGCG is active against many unrelated viruses that bind to heparan sulfate (HS) (i.e., HSV-1, HCV, vaccinia virus, Sindbis virus, adenovirus) or to sialic acid (SA) (i.e., IAV, reovirus), with IC50 in the micromolar range. EGCG acted directly on the virions and interacted with virion glycoproteins, without affecting the fluidity or integrity of virion envelopes. EGCG inhibited the attachment of the HS-binding HSV-1, HCV, vesicular stomatitis virus, vaccinia virus and adenovirus, and the SA-binding IAV and reovirus. EGCG competed with heparan sulfate for the binding of HSV-1 and HCV virions in heparin affinity chromatography. EGCG also competed with sialic acid for binding of IAV virions, in hemagglutination assays. Therefore, EGCG inhibits attachment by disrupting the low-affinity interactions between the virion glycoproteins and cellular HS- or SA-containing glycans. In summary, we have identified the first broad-spectrum inhibitor of viral attachment active against viruses that bind to glycosaminoglycans or to sialoglycans. These proof-of-principle findings open the possibility to develop small molecule antivirals with broad-spectrum activity against viral attachment of many unrelated human viruses.
Abstracts

114 A Combined Cell Based and Site Directed Mutagenesis Approach Defines Highly Conserved Residues Involved in the Selective Inhibition of the HIV-1 Ribonuclease H Function by Diketoacid Derivatives

Angela Corona1, Sandro Cosconati2, Sylvain Thierry3, Francesco S. Di Leva4, Olivier Delelis3, Francesca Esposito1, Roberto Di Santo5, Enzo Tramontano1
1University of Cagliari, Cagliari, Italy, 2DiSTABiF, Università Napoli 2, Caserta, Italy, 3LBPA, ENS, Cachan, France, 4Italian Institute of Tecnology, Genoa, Italy, 5La Sapienza University, Rome, Italy

Ribonuclease H (RH) activity of HIV-1 reverse transcriptase (RT) is an essential viral function that catalyzes highly specific hydrolytic events on the RNA strand of the RNA/DNA replication intermediate. Despite this critical role, RH is a promising drug target for which no inhibitor is available for treatment yet. In fact, the open morphology of the RH active site area makes difficult to individuate a druggable pocket. Diketoacid derivatives (DKA) are Mg chelating agents reported to be active site inhibitors of both HIV-1 RH and Integrase (IN) activities. The prototype DKA RDS1643 has been further developed into a series of ester and acid DKAs used as chemical tool to explore their binding region on RT and to demonstrate their mechanism of action in cell culture. Among the compounds tested on both RH and IN functions, most of ester derivatives showed selectivity for HIV-1 RH versus IN, while acids inhibited both functions and more potently IN. Molecular modeling and site-directed mutagenesis on RH domain showed a different binding pose for ester and acid DKAs, and proved that DKAs interact with residues not involved in the catalytic motif (R448, N474, Q475, Y501, R557). Noteworthy, Q475, N474 and Y501 are highly conserved residues part of the RH primer grip motif. In particular, ester derivative RDS1759 selectively inhibited RH activity and viral replication in the low micromolar range, making contacts with residues Q475, N474 and Y501 in the RH domain. Quantitative PCR studies and FACS analysis showed that RDS1759 selectively inhibits reverse transcription in cell-based assays. Overall, we provide the first demonstration that RH inhibition by DKAs is related not only to their chelating properties, but also to specific interactions with highly conserved amino acid residues in the RH domain, leading to effectively target HIV reverse transcription in cells, and hence offering important insights for rational optimization of RH inhibitors.

115 Dioxolane L-Nucleoside Analogue, L-BHHDU, Inhibits VZV Replication by Depleting the Cellular dTTP Pool

Chandrav De1, Uma S Singh2, Chung K Chu2, Fred Hagen3, Jennifer F Moffat1
1SUNY Upstate Medical University, Syracuse, USA, 2University of Georgia, Athens, USA, 3University of Rochester Medical Center, Rochester, USA

New antiviral drugs for varicella-zoster virus (VZV) with increased potency are needed, especially to prevent post-herpetic neuralgia. We found that L-bromovinyl uracil nucleoside analogue, L-BHDDU, was effective against VZV in culture and in a mouse model. The mechanism of action of L-BHDDU and its effect on drug-drug interactions was not known. Given its similar structure to brivudine (BVdU), we determined if L-BHDDU, like BVdU, inhibits 5-fluorouracil (5FU) metabolism. BALB/c mice were treated with 5FU alone or in combination with L-BHDDU and BVdU. After 60 mins of treatment, plasma was isolated and the relative 5FU concentration in blood was measured by LC MS/MS. L-BHDDU did not interfere with 5FU metabolism, suggesting that L-BHDDU is a safer drug than BVdU. However, L-BHDDU antagonized the activity of acyclovir (ACV), BVdU and foscarin in cultured cells. The observed antagonism of L-BHDDU with BVdU and ACV was due to the competition for phosphorylation by VZV thymidine kinase. To understand the mode of action of L-BHDDU, we asked whether addition of nucleosides reverses L-BHDDU inhibition of VZV in dividing and quiescent skin fibroblasts (HFFs). At concentrations 100-fold excess relative to L-BHDDU, thymidine and uridine, but not purines, restored VZV replication only in dividing HFFs, suggesting that L-BHDDU is a safer drug than BVdU. However, L-BHDDU antagonized the activity of acyclovir (ACV), BVdU and foscarin in cultured cells. The observed antagonism of L-BHDDU with BVdU and ACV was due to the competition for phosphorylation by VZV thymidine kinase. To understand the mode of action of L-BHDDU, we asked whether addition of nucleosides reverses L-BHDDU inhibition of VZV in dividing and quiescent skin fibroblasts (HFFs). At concentrations 100-fold excess relative to L-BHDDU, thymidine and uridine, but not purines, restored VZV replication only in dividing HFFs, suggesting that L-BHDDU activity is linked to pyrimidine metabolism. The cellular dNTP pool in infected and L-BHDDU treated human epithelial cells (ARPE-19) was measured by LC MS/MS. VZV infection increased the thymidine triphosphate (dTTP) pool by 6-fold compared to uninfected cells. There was a 4-fold reduction in the dTTP pool when infected cells were treated with L-BHDDU. Thus, L-BHDDU inhibits VZV replication by depleting the cellular dTTP pool. It is possible that L-BHDDU monophosphate decreases the dTTP pool by inhibiting thymidylate synthase (TS) enzyme, since we found a 2-fold increase in deoxyuridine monophosphate (dUMP) in treated cells, which is a substrate of TS. Studies are underway to test the effect of L-BHDDU monophosphate on cellular and viral TS.
116 Cell Kinase Inhibitor Panel Reveals Multiple Targets to Prevent Replication of Varicella-Zoster Virus

Bryan E. Bunnell, Dongmei Liu, Jennifer F. Moffat

1SUNY Upstate Medical University, Syracuse, USA, 2Syracuse University, Syracuse, USA

The alphaherpesvirus that causes chicken pox and shingles, varicella-zoster virus (VZV), infects skin fibroblasts and keratinocytes. These cells are typically quiescent and it is known that VZV manipulates the intracellular environment to activate MAPK signaling cascades, cell cycle regulators, and many transcription factors for its replication. We hypothesized that inhibition of cell kinases would prevent VZV replication and also elucidate which pathways are most important. We evaluated 80 kinase inhibitors for cytotoxicity and anti-proliferative effects on human foreskin fibroblasts, and then determined their antiviral efficacy against VZV-ORF9-Luc strain. Sixteen kinase inhibitors were identified that were noncytotoxic at < 25 µM and prevented VZV replication at < 12 µM. Receptor tyrosine kinases (EGFRK, NGFRK and PDGFRK) were pivotal for VZV replication and 6 potent compounds inhibited these targets (Tyrphostins 9, 23, AG1478, AG879, RG1462). Their common downstream substrates were also found to be necessary for VZV replication: protein kinase A (PKA, H-89•2HCL), Akt (PKB, Triciribine), protein kinase C (PKC, GF109203X and PKC412), and Bruton’s tyrosine kinase (Btk, Terreic acid). Other potent compounds blocked the activity of calmodulin-dependent protein kinase (CaMKII, KN-93 and KN-62) and glycogen synthase kinase 3 beta (GSK-3b, Indirubin-3’-monooxime and Kenpaullone). We also identified two compounds with known antiviral activity, the cyclin-dependent kinase inhibitors Roscovitine and N9-isopropylolomoucine. Thus VZV infection causes changes in the intracellular environment that expose antiviral targets in multiple pathways. Inhibition of receptor tyrosine kinases, which initiate these pathways, is an area of intense drug discovery efforts for molecular oncology.

117 Biosynthesis and Degradation of the Triphosphates of Cyclopropavir and Ganciclovir in Human Cytomegalovirus Infected Cells

Brian G Gentry, John C Drach

1Drake University College of Pharmacy and Health Sciences, Des Moines, USA, 2University of Michigan School of Dentistry, Ann Arbor, USA

Human cytomegalovirus (HCMV) is a widespread pathogen that can cause severe disease in immunologically immature and immunocompromised patients. Current pharmacotherapies for the treatment of systemic HCMV include ganciclovir (GCV), cidofovir, and foscarnet. However, high incidence rates of adverse effects are prevalent and limit the use of these drugs. Cyclopropavir (CPV) is 10-fold more active against HCMV in vitro when compared to GCV (EC50’s = 0.46 and 4.1 µM, respectively) without any observed increase in cytotoxicity. We have previously determined that the viral protein kinase pUL97 and endogenous cellular kinases are responsible for the conversion of CPV to a triphosphate, the active compound responsible for inhibiting viral DNA synthesis and viral replication. We now report this conversion in HCMV-infected cells. We incubated HCMV-infected cells with equivalently effective concentrations (~5 times the EC50) of either CPV (2.5 µM) or GCV (25 µM) and observed a time dependent increase in triphosphate levels for both compounds (CPV-TP = 121 ± 11 pmol/10^6 cells, peak at 120 hrs GCV-TP = 43.7 ± 0.4 pmol/10^6 cells, peak at 96 hrs). Incubation with only an EC50 concentration of CPV (0.46 µM) resulted in proportionately less CPV-TP and gave an intracellular concentration of CPV-TP of 5.0 ± 0.83 µM. A longer half-life was observed for GCV-TP (48.2 ± 5.7 hrs) compared to CPV-TP (23.8 ± 5.1 hrs). Since the administration of CPV resulted in greater biosynthesis of triphosphate when compared to GCV but with a shorter half-life, data were combined into single plots and areas under the curve were calculated to determine which combination of properties resulted in the greater exposure of HCMV-infected cells to active compound. The area under the curve for CPV-TP was 8680 ± 930 pmol-hours/10^6 cells, approximately two fold greater than the area under the curve for GCV-TP of 4520 ± 420 pmol-hours/10^6 cells. We conclude that the more potent activity of CPV is due to the greater amount of CPV-TP produced in HCMV-infected cells when compared to the biosynthesis of GCV-TP from GCV.
The Discovery and In Vitro Characterization of Pyrido-Pyrimidinone Antiretrovirals with Selective Activity against HIV Ribonuclease H as well as Dual Inhibitors of both Ribonuclease H and Integrase

Peter Gerondelis, John W. Seal III, Derek J. Parks, Kendra E. Hightower, Kevin W. Brown, Robert G. Ferris, Emile J. Velthuisen, Brian A. Johns

GlaxoSmithKline, Research Triangle Park, USA

The polymerase and RNase H active sites of HIV reverse transcriptase (RT), both require divalent metal for catalysis. In an effort to identify novel competitive inhibitors of either active site, an RT strand transfer assay was developed to run under extremely low dNTP concentrations while maintaining the RNase H-mediated, strand transfer as the rate limiting step. Focused screening of a library of metal binding scaffolds led to the discovery a variety of starting points that appeared selective for the inhibition of RNase H, some of which also exhibited activity against the viral integrase (IN) which also requires divalent metal. Here we report on the discovery and characterization of a series of novel, pyrido-pyrimidinone-based antivirals and the resolution and confirmation of their MOA. Initial MOA resolution was established with enzymatic assays developed for the ultra-sensitive resolution of activity against both the viral and human RNase H enzymes as well as for the RT polymerase activity. Activity against RT and IN in infected cells was determined by molecular techniques in addition to profiling against viral variants known to be resistant to existing POL and IN inhibitors. As a result of these efforts, potent and selective inhibitors of HIV RNase H were resolved. In addition, a sub-series of compounds with dually potent activity against both RNase H and IN were identified as well. To confirm these findings, we selected for viral variants, in the presence of selective RNase H inhibitors, that harbored novel coding changes in RT but not IN. In addition, selection of coding changes in both RT and IN was achieved with a compound that exhibited potent activity against both RNase H and IN. For the latter, selection of variants were slower to arise consistent with the notion that it possesses an intrinsically higher barrier to resistance than single MOA agents.

STING Agonist Induces A Potent Innate Antiviral Immune Response Against Hepatitis B Virus

Fang Guo¹, Fei Liu¹, Xuesen Zhao¹,², Timothy M. Block¹,², Ju-Tao Guo¹, Jinhong Chang¹

¹Drexel University College of Medicine, Doylestown, USA, ²Baruch S. Blumberg Institute, Hepatitis B Foundation, Doylestown, USA

Chronic hepatitis B virus (HBV) infection is due to the failure of a host to mount a sufficient immune response to clear the virus. Accordingly, restoration of innate as well as adaptive antiviral responses against HBV has been considered as a curative therapeutic approach for chronic hepatitis B. In our efforts toward discovering small molecular agonists of pattern recognition receptors and evaluating their antiviral activities against HBV, we found that 5,6-Dimethylxanthenone-4-acetic acid (DMXAA), an agonist of mouse stimulator of interferon genes (STING), induced a robust cytokine response in macrophages, which in turn potently suppressed HBV replication in mouse hepatocytes by reducing the amount of cytoplasmic viral capsids. Mechanistic studies further demonstrated that the DMXAA-induced antiviral response is indeed STING-dependent and primarily mediated by type I interferons. However, other cytokines, including tumor necrosis factor alpha, may also play a role. Profiling of cytokines induced by DMXAA and agonists of representative toll-like receptors in mouse macrophages revealed that unlike TLR agonists that induced a predominant inflammatory cytokine and chemokine responses, the STING agonist induced a more specific cytokine response dominated by type I interferons. Our studies have thus proved the concept that activation of STING pathway induces a strong antiviral response against HBV and development of small molecule human STING agonists as potential curative therapeutics of chronic hepatitis B is warranted.
120 Antiviral Activity of Vemurafenib Against Influenza A Virus in Mice and Cells with Wild Type BRAF – Indication of a New Mode of Action

Emanuel Haasbach1, Carmen Hartmayer1, Stefanie Hildenbrand1, Stephan Ludwig2, Oliver Planz1
1 University of Tuebingen, Tuebingen, Germany, 2 University of Muenster, Muenster, Germany

Vemurafenib is a Raf-kinase inhibitor that has remarkable antitumor activity in melanoma patients with a BRAF V600E mutation but not in melanomas with wild type BRAF. In our previous work we were able to demonstrate that influenza virus replication is dependent on the Raf/MEK/ERK signaling pathway and that inhibition of this pathway with specific inhibitors leads to reduction of viral titer. Raf- and MEK-inhibitors interfere with the Ras-dependent Raf/MEK/ERK1/2 signaling pathway, which is a major regulator of cell proliferation and survival. Hyperactivation of this pathway is frequently observed in human malignancies as a result of aberrant activation of receptor tyrosine kinases or gain-of-function mutations in RAS or RAF genes. Components of the ERK1/2 pathway are therefore viewed as attractive candidates for the development of targeted therapies of cancer and substances inhibiting Raf or MEK are in current state of clinical evaluation in cancer. We found that these Inhibitors have antiviral activity against influenza virus with IC50 levels in the nM range. Vemurafenib is highly active against influenza virus in A549 cells. Western Blot analyses revealed that Vemurafenib treatment does not result in inhibition of the Raf/MEK/ERK signaling pathway due to the fact that this inhibitor is only active in cells harboring Raf with a V600E mutation. In contrast, Vemurafenib treatment leads to activation of the Raf/MEK/ERK pathway. The fact that cell proliferation is not inhibited after Vemurafenib treatment of A549 cells further supports the observation that the inhibitor is not able to inhibit ERK. Vemurafenib shows also antiviral activity in C57BL/6 mice with wild type Raf. This clearly supports our view that antiviral activity of Vemurafenib is independent of Raf/MEK/ERK signaling pathway inhibition. Thus, the question arises what mode of action is responsible for the antiviral activity of Vemurafenib. Here, we will demonstrate the role of Ca2+ after Vemurafenib treatment and the influence of Ca2+ for virus replication. We will present for the first time a new mode of antiviral action against influenza virus.

121 Reserve Autophagic Capacity in Alveolar Epithelia Provides a Replicative Niche for Influenza A Virus

David R Hahn, Cheng-Lun Na, Timothy E Weaver
Perinatal Institute, Section of Neonatology, Perinatal and Pulmonary Biology Cincinnati Children’s Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, USA

Autophagy contributes to cellular homeostasis through metabolite recycling and degradation of cytotoxic protein aggregates and damaged organelles. While recent studies have established that the requirement for basal autophagy is largely tissue specific, the importance of autophagy for alveolar epithelial cell homeostasis remains an important knowledge gap. In the present study we generated two mouse models, with >90% or >50% recombination at the Atg5 locus in the distal respiratory epithelium, to assess the effect of dose-dependent decreases in autophagy on alveolar homeostasis and influenza A pathogenesis. A 90% decrease in autophagy was well tolerated in young adult mice but resulted in alveolar septal thickening and altered lung mechanics in aged animals, consistent with accumulation of damage over time. By comparison, a 50% decrease in autophagy had no effect on alveolar structure or function throughout the murine life span, indicating that basal autophagy in this compartment exceeds that required for homeostasis. Importantly, a 50% decrease in autophagy in the bronchoalveolar epithelium significantly attenuated influenza A/H3N2 viral replication, leading to improved lung structure and function and reduced morbidity and mortality following infection. The reserve of autophagic capacity in the alveolar epithelium appears to provide a niche for replication of influenza A virus, and may represent an antigen-independent target for future antiviral therapy.
RASD1: A Novel Gene Target of HSV-2
Susan C Irvin\textsuperscript{1}, Natalia Cheshenko\textsuperscript{1}, Viviana Simon\textsuperscript{2}, Betsy C Herold\textsuperscript{1}
\textsuperscript{1}Albert Einstein College of Medicine, Bronx, USA, \textsuperscript{2}Mount Sinai School of Medicine, New York, USA

Herpes simplex virus (HSV) is the leading cause of genital disease, a major risk factor for HIV and the most common cause of neonatal encephalitis. Acyclovir and related produgs are the only approved therapeutics, but do not prevent latency or reactivation and drug resistance is a clinical problem. Therefore, it is imperative that novel targets for drug development be identified. We conducted a microarray to identify cellular genes regulated by HSV that might provide new targets for drug development. Human vaginal epithelial cells (VK2/E6E7) were exposed to HSV-2(G) or UV-inactivated virus and RNA was extracted 30 minutes and 6 hours post-infection two independent experiments were conducted each in triplicate. We focused on RASD1, which was upregulated 3.5-fold in both microarray assays at 6 h pi, following exposure to live but not UV-inactivated virus. The upregulation was confirmed by qRT-PCR (mean fold 408 +/- 131 SEM) and Nanostring technology (36-fold). RASD1 was also upregulated in primary monocyte-derived dendritic cells (moDCs) (600-fold) as determine by Nanostring. Notably HIV had no impact on RASD-1 expression in either moDCs or VK2/E6E7 cells, suggesting that the response may be HSV specific. To determine if RASD1 contributes to infection, VK2/E6E7 were transfected with RASD1 or control siRNA. Silencing of RASD1 resulted in a significant reduction in viral plaque number and size, suggesting RASD1 facilitates HSV propagation. Little is currently known about RASD1 and no relationship with viral infection has been previously identified. RASD1 is a member of the Ras superfamily and is expressed in brain, heart, liver, pituitary, and reproductive organs. RASD1 participates in the nitric oxide (NO) pathway and can inhibit signal transduction to ERK1/2. Future studies will examine RASD1 expression in biopsies obtained from HSV infected subjects and controls, determine the role RASD1 plays in infection and the mechanism by which virus induces its upregulation. We speculate that HSV-RASD1 interactions may provide a novel target for new antiviral drug development.

SB 9200, a Novel Anti-HBV Agent–In Vitro Combination Studies and Pharmacodynamic Studies in Woodchucks
R.P. Iyer\textsuperscript{1}, A. Sheri\textsuperscript{1}, R.K. Pandey\textsuperscript{1}, S. Padmanabhan\textsuperscript{1}, J.K. Marquis\textsuperscript{1}, J.M. Skell\textsuperscript{1}, B.E. Korba\textsuperscript{2}, J.D. Morrey\textsuperscript{3}
\textsuperscript{1}Spring Bank Pharmaceuticals, Milford, USA, \textsuperscript{2}Georgetown University Medical Center, Washington, USA, \textsuperscript{3}Institute for Antiviral Research, Utah State University, Logan, USA

BACKGROUND: Over 350 million people worldwide are chronically infected with hepatitis B virus (HBV). While prolonged therapy with direct acting antivirals can effectively suppress viral replication, the underlying liver disease continues to progress to hepatocellular carcinoma. New antiviral agents that have the potential to eliminate cccDNA and effect viral clearance are urgently needed. SB 9200 is a nucleotide compound with a novel mechanism of action involving activation of the cytosolic sensors, RIG-I and NOD2 that shows potent antiviral activity against wild type-, and resistant variants of HBV. In previous studies, orally administered SB 9200 had shown strong antiviral activity in the HBV transgenic mouse model. Tissue distribution studies using radiolabeled SB 9200 revealed significant compound distribution to the liver. As part of further preclinical development, synergy studies of SB 9200 with Tenofovir and Entecavir were carried out. To further establish its in vivo antiviral activity, pharmacodynamic studies of orally administered SB 9200 in chronically WHV-infected woodchucks were performed.

METHODS: In vitro combination studies of SB 9200 with Tenofovir and Entecavir were done at molar ratios expected to give equipotent antiviral activity using chronically infected HepG2.2.15 cells. For pharmacodynamics evaluation, three treatment groups each of four chronically infected woodchucks were administered SB 9200 in liquid diet at 9, 15, and 25 mg/kg/day for four weeks with follow up until week 16 of the study. Blood samples were drawn at designated days prior to, during, and after the drug administration, for WHV DNA analysis and serological testing. Serum WHV DNA levels were measured by dot blot hybridization.

RESULTS: SB 9200 was found to have synergistic anti-HBV activity when combined with Tenofovir and Entecavir. Oral dosing of woodchucks with SB 9200 was well tolerated, with no signs of overt toxicity and dose-dependent declines in serum WHV DNA were seen. SB 9200 is being advanced for clinical development against HBV.
124 Genetic Vaccine Constructed with Hantavirus Gn Targeting to MIIC by lysosome-associated Membrane Protein, Conferred BALB/c Mice Satisfying Immune Protection Against HTNV Infection
Dongbo Jiang, Yuanjie Sun, Linfeng Chen, Gefei Zhang, Fanglin Zhang, Kun Yang
Fourth Military Medical University, Xi’an, China

Lysosome-associated membrane protein (LAMP) can target to endosome/lysosome, one of the most important components of the MHC class II-processing compartment (MIIC) in the exogenous antigen-processing pathway. LAMP targeting could greatly enhance the immune response and DNA plasmids encoding endogenous antigen could take advantage of LAMP and be carried directly into MIIC activating Th for effective immune response and long-term immune memory. Hantavirus glycoprotein N-terminal, named Gn, could induce neutralizing antibody production with a low serum titer as natural infection. To develop and analyze a novel effective vaccine against HTNV, we constructed three eukaryotic vectors as naked DNA vaccine named pVAX-Gn, pVAX-LAMP and pVAX-LAMP/Gn, respectively. Balb/c mice were immunized with those plasmids, the specific humoral and cellular responses elicited against HTNV Gn were measured by ELISA, cytotoxicity assays and ELISPOT assay (IFN-γ). To measure the protective efficacy, virus challenging in vivo and neutralizing antibody valence were conducted by viral load detection (qRT-PCR and sandwich ELISA) and the cell microculture neutralization test. We found that HTNV Gn showed a strong immunogenicity to elicit both humoral and cellular responses with LAMP as a chimera. Compared with the current prophylactic inactive vaccine, both pVAX-Gn and pVAX-LAMP/Gn showed a stronger cellular response. Being worth raising, a significant long term memory response was observed only for the group of pVAX-LAMP/Gn. Histopathological analysis by HE staining demonstrated that pVAX-LAMP/Gn was not harmful. Results of protection assay in vivo indicated that the immune response established was HTNV specific and protective. These findings not only demonstrated that the LAMP as a trafficking molecule can introduce Gn to MHCII presenting pathway and significantly enhanced HTNV specific immune response, but also suggested that the pVAX-LAMP/Gn as a DNA vaccine had potential application on clinic for HTNV infection immunoprophylaxis.

125 Efficiency of Incorporation and Chain Termination Determines the Inhibition Potency of 2’-Modified Nucleotide Analogs against HCV Polymerase
Zhinan Jin, Amy Fung, Natalia Dyatkina, Guangyi Wang, Leo Beigelman, Jerome Deval
Alios BioPharma Inc., South San Francisco, USA

BACKGROUND: Ribonucleotide analog inhibitors of the RNA-dependent RNA polymerase of hepatitis C virus represent one of the most exciting recent developments in anti-HCV antiviral therapy. Although it is well established that these inhibitors act by competing with natural NTP substrates to be incorporated into the elongating RNA molecules and causing chain termination, strategies to rationally optimize antiviral potency based on enzyme kinetics remain elusive.

METHODS AND RESULTS: We developed a simple method to measure the single nucleotide incorporation efficiency ($k_{pol}/K_d$) of nucleotide analogs by HCV polymerase elongation complex using pre-steady state kinetics derived from only a single time point. The new method was validated by computer simulation and single nucleotide incorporation assays. From our kinetic measurements, we found that substitutions at the 2’-position on the ribose can greatly affect the incorporation efficiency of UTP analogs by HCV polymerase, with a preference ranking from OH>F>NH$_2$>C-Me>ara>N$_3$. We also measured the $k_{pol}/K_d$ of the next correct nucleotide incorporation to the analog terminated RNA to determine the extent of the chain termination caused by a UTP analog. Interestingly, the extent of chain termination followed a different order, with only 2’C-Me- and 2’ara-UTP being able to cause complete and immediate chain termination. The overall inhibition potency (IC$_{50}$) of these UTP analogs was measured under steady-state kinetic conditions of multiple nucleotide incorporations. We found no correlation between substrate efficiency and overall inhibition potency across all molecules, which could be explained by the different chain termination profile of each UTP analog.

CONCLUSIONS: We provide the first attempt to use pre-steady state kinetics to understand the mechanism of action of 2’-modified NTP analogs against HCV polymerase. These efforts show that efficiency of chain termination is the primary determinant that drives the inhibition potency of nucleotide analogs. Overall, these results should help to rationally design new nucleotide analogs to inhibit HCV replication.
126 Down Regulation of M2 Gene and Inhibition of Influenza Virus Replication in Host Cells Using Catalytic Nucleic Acid Enzymes

Madhu Khanna, Binod Kumar, Roopali Rajput, Latika Saxena
Department of Respiratory Virology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India

Pervasive stigma has surrounded the whole world since the beginning of the recent pandemic as influenza viruses still continue to pose serious health emergencies leading to enormous socio-economic loss. Influenza A virus genome segment 7 encodes protein M2, which is the matrix 2 protein playing crucial role in the virus life cycle. Any antiviral strategy that aims at reducing, in particular, the expression of this genome segment should, in principle, reduce the infectivity of the virus. We developed a specific antiviral approach at the molecular level and designed several novel 10-23 DNAzymes (Dz) specifically targeted to cleave at the conserved domains of the influenza virus M2 RNA. We observed that the Mg(2+)-dependent sequence-specific cleavage of M2 RNA was achieved by the designed Dz in a dose-dependent manner. RT-PCR and real-time RT-PCR assays showed significant 65% inhibition of target gene upon specific Dz treatment. The transfection of MDCK cells with Dz showed reduced cytopathic effect caused by influenza A virus (A/PR/8/34-H1N1) and considerably reduced the M2 protein expression. This catalytic potential of Dz, in principle, resulted in more effective gene suppression, inhibited the whole virus replication in host cell, and thus could be exploited for therapeutic purposes.

127 Inhibition of Influenza B virus M1 Protein by 3H,3’H-spiro[benzofuran-2,1’-isobenzofuran]-3,3’-dione

Meehyein Kim, Ye Jin Jang, Yun Young Go, Chonsaeng Kim, Yashwardha Malpani, Young-Sik Jung, Chong-Kyo Lee
Division of Drug Discovery Research, Korea Research Institute of Chemical Technology, Daejeon, South Korea

In our previous report, it was suggested that the spiro compound 3H,3’H-spiro[benzofuran-2,1’-isobenzofuran]-3,3’-dione, named 3b, inhibited specifically influenza B virus infection into Madin Darby canine kidney (MDCK) cells with half-maximal effective concentration values between 3.0~16.1 mM and half-maximal cytotoxic concentration values above 500 mM. The present study is focused on investigation of its antiviral mechanism of action. Time-of-addition assay and viral polymerase activity analysis showed no influence of 3b on virus entry or viral RNA replication. Notably, in a confocal microscopy it was observed that nuclear export of viral NP protein was delayed in the presence of 3b at 10 h postinfection (p.i.) and at a later time point, 24 h p.i., clusters of aggregated NP protein was found in cytoplasm. To identify a protein targeted by 3b, its resistant viruses were induced by repeated infection of influenza virus B/Panama/45/90 into MDCK cells. After 17 passages, the virus was three-fold less sensitive to 3b than the original one. We analyzed the full viral genome sequences and observed 100% mutation at a single amino acid of M1 protein, which is one of key proteins for nuclear export of vRNP complex at a late stage of virus infection. Our data show that the spiro compound 3b is an efficient M1 inhibitor, which could serve as lead compound for the discovery of a novel influenza virus inhibitor.
128 Structure-Based Drug Design of Novel Active Site and Allosteric HIV-1 RNase H Inhibitors
Karen A. Kirby¹, Hilary A. Schmidt¹, Jing Tang¹, Tatiana Ilina⁴, Qiongying Yang¹, Zhengqiang Wang³, Michael A. Parniak⁴, Stefan G. Sarafianos¹,²,⁵
¹Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, USA, ²Department of Molecular Microbiology & Immunology, University of Missouri School of Medicine, Columbia, USA, ³Center for Drug Design, University of Minnesota, Minneapolis, USA, ⁴Department of Microbiology & Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, USA, ⁵Department of Biochemistry, University of Missouri, Columbia, USA

The reverse transcriptase (RT) enzyme of HIV-1 plays a critical role in viral replication, using both polymerase and RNase H (RNH) functions to convert the single-stranded RNA viral genome into double-stranded DNA for subsequent integration into the host genome. All currently FDA-approved drugs against RT only target the polymerase function of this enzyme. Because many patients develop resistance to these drugs, it is imperative to identify new drug candidates directed at HIV-1 targets not addressed by current therapeutics, as these are likely to be effective against current drug-resistant HIV-1 strains. The RNH activity of RT is the last enzymatic function of HIV-1 yet to be targeted by approved therapies and is therefore an attractive antiviral target. Our multidisciplinary team is using a structure-based design approach to improve existing leads and develop new RNH inhibitors (RNHIs) with increased potency. We have solved the crystal structures of HIV-1 RT in complex with a 1,2,4-triazole-based RNHI (7390) and also in complex with two different RNHIs simultaneously (7390 and JT-8-169, a hydroxypyrimidinone-based RNHI). The 7390 1,2,4-triazole acts as a long-range allosteric RNHI by binding at the nonnucleoside RT inhibitor (NNRTI) binding pocket, and the JT-8-169 hydroxypyrimidinone blocks RNH activity by binding at the RNH active site. These crystal structures provide the molecular details of two different modes of RNH inhibition. Information gained from these structures should help guide design efforts based on these scaffolds that will potentially give rise to new inhibitors with enhanced antiviral potency.

129 MAPKAP Kinase 3 (MK3) Suppresses Ifn-gamma Gene Expression and Attenuates NK Cell Cytotoxicity and Th1 CD4 T Cell Development in influenza A Virus Infected Mice
Katharina Koether¹, Carolin Nordhoff¹, Jay H. Bream², Matthias Gaestel³, Viktor Wixler¹, Stephan Ludwig¹
¹Institute of Molecular Virology, Muenster, Germany, ²Johns Hopkins Bloomberg School of Public Health, Baltimore, Germany, ³Hannover Medical School, Hannover, Germany

Virus-activated cellular kinases have been shown to be promising targets for a novel strategy of cell-directed antiviral intervention against influenza A viruses (IAV). MK2 and MK3 are downstream targets of MAP kinases p38 and ERK1/2 and confer, among other functions, the mRNA stability of several inflammatory cytokines, including TNF-α and IL-10. While MK2 is expressed ubiquitously, the expression of MK3 is restricted to muscle, liver, heart T and NK cells. Using MK deficient and WT mice, we could demonstrate an inhibitory effect of MK3, but not MK2, on IFN-γ expression in T and NK lymphocytes. We provide evidence that the inhibitory effect of MK3 is based on negative feedback phosphorylation of p38 and ERK1/2, which results in a decreased binding of Stat4 to the IFN-γ promoter and in a reduced expression of IFN-γ mRNA and protein. Consequently, all Mk3-/- mice challenged with the Th1-inducing IAV survived the WT LD50 virus doses. The reduced disease severity in Mk3-/- mice was accompanied by a more than ten-fold reduced viral lung titers as well as by increased numbers of activated NK cells and enhanced Th1 activation of CD4 T cells. Our data describe the protein kinase MK3 as a novel regulator of the innate and adaptive immune responses. Thus, specific inhibitors of MK3 may serve as immune modulating agents that promote a IFN-γ governed Th1 immune response thereby protecting mice from a lethal IAV challenge.
Generation and Functional Characterization of Human Anti-V3 scFv Antibodies Against HIV-1 clade C Viruses.
Rajesh Kumar1, Ruchi Kumari1, Raiees Andrabi1, Ashutosh Tiwari1,3, Hilal Ahmed1, Lubina Khan1, Subrata Sinha1,2, Kalpana Luthra1
1Department of Biochemistry, New Delhi, India, 2National Brain Research Centre, Manesar, India, 3Translational Health Science and Technology Institute (THSTI), Gurgaon, India

The third variable region (V3) is highly conserved and crucial target on gp120 for neutralizing antibodies, primarily due to its involvement in co-receptor (CXCR4 or CCR5) binding. Two anti-V3 scFvs, 1E7B and F2C were generated from an antigen specific phage library after four round of biopanning. The scFv gene was cloned into the pAK400 expression vector, and 6His-tagged scFv antibodies expressed in periplasm of Escherichia coli HB2151, which were then purified by nickel affinity chromatography. Characterization of these scFv clones by SDS-PAGE and Western blot showed that the scFv monoclonals were ~32kDa. The functional activity of these scFv monoclonals were checked by indirect ELISA. The purified scFv protein showed specific binding to V3 antigen and did not show any reactivity against other unrelated peptides. The two scFv clone showed varying degrees of neutralization against clade A, B and C viruses. The scFv clone F2C was able to neutralize tier 1 viruses from clade B & C while 1E7B was able to neutralize both tier 1 and more resistant tier 2 viruses from different clades. The two scFv 1E7B and F2C clone exhibit IGHV4-31*03 and IGHV4-31*02 gene usages in heavy chain and IGKV3-20*01 and IGKV2-28*01 in light chain respectively. Our study suggests that the anti-V3 scFv derived from subtype-C infected Indian patients display neutralization potential against both tier 1 & 2 viruses. Further defining the epitope specificities of these anti-V3 scFvs will be helpful in identification of neutralization epitopes that are conserved within the different HIV clades as well as those that are unique to Indian clade C viruses, prerequisite for designing a polyvalent vaccine against a broad spectrum of HIV-1 isolates.

Fast HCV RNA Elimination and NSSA Redistribution by Daclatasvir
Dandan Liu1, Juan Ji1, Tanya P. Ndongwe1, Charles M. Rice2, Robert O. Ralston1, Stefan G. Sarafianos1
1University of Missouri-Columbia, Columbia, USA, 2The Rockefeller University, New York, USA

Hepatitis C virus (HCV) infections result in more than 350,000 deaths from cirrhosis and hepatocellular carcinoma. In the US, >3 million people are chronically infected by HCV and ~15,000 die from HCV-related liver disease. While earlier therapeutic strategies relied exclusively on host-targeting antiviral agents (HTAs) interferon and ribavirin, a number of direct acting antiviral agents (DAAs) have been recently approved, aiming for an interferon-free strategy with short treatment duration and fewer side effects. Daclatasvir (DCV) is a highly potent DAA that targets non-structural protein 5A (NSSA). DCV treatment leads to a rapid decline of extracellular HCV titers, consistent with its proposed effect on both viral replication and assembly/secretion. To facilitate studies on the mechanism of action (MOA) and efficacy of DCV and other DAA/HTAs, we used a multiplex assay system, which uses RT-PCR, flow cytometry, western blot analysis, a Gaussia luciferase reporter system, and a novel image profiling assay that follows the NSSA redistribution in response to drug treatment. This multi-prong approach allows detailed characterization of the kinetics and MOA of DAAs from various families. We used these assays to examine which DAA exerts its inhibitory effect faster. We evaluated an NSSA inhibitor (DCV), an NS3/4A inhibitor (danoprevir) and an NS5B inhibitor (sofosbuvir). While all assays provided reliable estimation of HCV inhibition at later time points (24 hrs or more, post drug treatment), only the RT-PCR and image profiling analyses were able to clearly show drug effects at early time points (~8 hrs). Our data demonstrate that DCV has the fastest effect on redistribution of NSSA and on suppression of viral RNA. Our approach should strengthen our understanding of the biological processes involved in HCV replication, provide insights into MOA of DAA, and help identify optimal drug combinations.
132 Overview of Influenza Virus Infections in Kenya: Past, Present and Future

Duncan Matheka, Jolyne Mokaya, Marybeth Maritim
University of Nairobi, Nairobi, Kenya

The World Health Organization (WHO) estimates that acute lower respiratory infections account for 4 million deaths per year. The rates are even higher in developing countries. Influenza, a virus causing respiratory infections, has widely been studied in developed countries. However, there is paucity of data on its epidemiology, seasonality and burden in most developing countries. In the contrary, Kenya (a developing country) has an elaborate national epidemiology-surveillance network for influenza, where a lot of data is generated on the epidemiology and seasonality of influenza in Kenya and the East African region. Several steps have been taken to control influenza in Kenya, including vaccination and surveillance programs. However, some challenges still exist. This article explores the pattern of influenza and existing interventions in Kenya, and highlights suggestions on what can be done to adequately control this virus in future.

133 Expression of Immunological Markers in Liver Tissue of HIV/HCV Co-infected Patients

Natallia V. Matsuieuskaya, Vladimir M. Tsyrykunov, Michail G. Zubritskiy, Nikolay I. Prokopchik
Grodno State Medical University, Grodno, Belarus

AIM OF THE STUDY: to evaluate expression of immunological and viral markers in HIV/HCV co-infected patients with different types of liver diseases progression.

MATERIAL AND METHODS. Antibodies: anti-HIV p24, anti-human CD8, anti-human CD56, anti-human HLA-DP, DQ, DR antigens, anti-Herpes Simplex Virus 1 and 2 types (Dako), anti-human TNF-α, anti-hepatitis C, NS4 HCV, anti-human CD184 antibodies (AbDserotec) were used. Expression of markers was evaluated by immunohistochemistry in paraffin liver tissue in 18 patients with HIV/HCV co-infection and in 15 patients with HIV infection.

RESULTS. In patients with HIV/HCV co-infection higher content of CD8+ cells and low CXCR4+ cells in liver inflammatory infiltrate (LII), higher content of HLA-DP, DQ, DR+ Kupffer cells, p24HIV+ hepatocytes (HP) and p24HIV+ Kuffer cells (KC) were detected in comparison with HIV-monoinfected patients (p<0.05). Productive HIV infection of HP (p24HIV+) associated with productive HCV-infection (NS4 HCV+) of HP, activation and increased expression of TNF-α in KC. In HIV/HCV co-infected patients rapid liver disease progression was associated with higher content of CD56+ and CD68+ cells in LII, higher activation (HLA-DP, DQ, DR+) of LII cells and also higher expression of HSV 1 and 2 antigens in HP and KC in comparison with patients with slow liver disease progression.

CONCLUSION. Therapeutic approaches for reduction of productive HIV and HCV infections of liver cells for decreasing of excessive immune activation and expression of opportunistic infections in the liver tissue should be used in HIV/HCV co-infected patients.

134 Statistical Analysis Reveals Parent-of-Origin Effects on Influenza Pathogenesis in a Diallel Cross of Mice

Paul L. Maurizio, Martin T. Ferris, Alan C. Whitmore, William Valdar, Mark T. Heise
University of North Carolina at Chapel Hill, Chapel Hill, USA

Influenza A viruses (IAVs) are rapidly evolving RNA viruses that are responsible for regular epidemics and periodic pandemics globally. Viral pathogenesis results from a complex interplay dependent upon both host and pathogen genetics. Here we describe a diallel study to estimate the broad host genetic architecture of IAV infection response, with the goal of using this information to design and prioritize further experiments identifying specific host factors contributing to variable disease responses. We propose an original statistical model and visualization scheme to characterize the effect of host genetic architecture on influenza disease outcomes. Because the diallel mice are derived from F1 crosses of the eight mouse strains used to generate the Collaborative Cross (CC), our findings are applicable to systems genetics platforms that recapitulate much of the genetic complexity of human populations. Our model incorporates sex, treatment, batch, cross, and interaction terms to generate individual and group-wise estimates of IAV severity. We present posterior predictive estimates of weight loss in IAV-infected and mock-infected mice over the course of
four days, and demonstrate the utility of Bayesian hierarchical modeling to precisely describe and stably predict complex genetic, sex and parent-of-origin effects on infection outcomes. Our results are validated by showing a prominent role of Mx1 in promoting resistance to influenza infection outcomes, and further show substantial treatment by cross effects and treatment by parent-of-origin effects, suggesting susceptibility to flu may be affected by whether chromosomes/alleles are inherited from a maternal or paternal line. This modeling approach is broadly applicable to other similarly derived pathogen datasets (e.g. Severe Acute Respiratory Syndrome (SARS) coronavirus) and has the potential to increase our understanding of epigenetic effects of host populations on viral disease severity.

135 The Impact of the IEC (information, education and communication) in the affidavit of sex workers: Study realized by the youth center Coulibaly sidiki of the University of Kinshasa (Democratic Republic of Congo)
Floribert M. MONGA1, Gaetan M. MUTOMBO2
1Free University of Kinshasa, KINSHASA, Congo-Kinshasa, 2Youth Center Coulibaly Sidiki, KINSHASA, Congo-Kinshasa

Sex workers (SW) constitute an important group in the transmission of HIV infection. Their sanitary and social follow-up impacts the prevention of HIV infection. The purpose of this study was to determine the impact of the IEC in the prevention of STI and HIV next to sex workers. This prospective and descriptive study was performed at the youth center Coulibaly sidiki of the University of Kinshasa on a period of 3 months (September to November 2012). Sex Workers in the socio-sanitary file of the center who came to make their medical visit during the period of study were enrolled in the study. They were questioned through mid-directive questions sheet, oriented for the following data: socio-demographic data, questions concerning the sanitary follow-up, on going of IEC sessions, the impact of IEC sessions, in end some open question was devoted to possibility suggestions of SW to increase their follow-up. Fifty two SW were included. The mean age was 35 years. The majority of SW was living at Kinshasa (75%). 62% were divorced; 24% single; 6, 5% widows. Among SW: 16 or 40% had minor children. More than the half of SW did not have any other professional activity. During the IEC sessions 87% of SW discussed: STI and HIV/AIDS (26%), police harassment (12, 5%); the solidarity between SW (16%). The frequency of STI was 65, 7% before the IEC sessions and 68% of SW ignored the type of STI. Systematic using of condom was 78, 5%. Concerning evaluation of their knowledge in the field of fighting against HIV and STI, acquired through IEC session: 30% knew the HIV transmission’s way, 14, 5% recognized easily a STI. SW are important in HIV and STI propagation because they constitute a gangway with the general population and the IEC remains one of the best strategies to prevent HIV and STI infections.

136 A Versatile in Vitro Assay Identifies Inhibitors and Stimulators of Nonsegmented Negative-sense RNA Virus Polymerase Function
Benjamin Morin1, Linda J. Rennick2, W. Paul Duprex2, Sean P. J. Whelan1
1Harvard Medical School, Boston, USA, 2Boston University School of Medicine, Boston, USA

The minimal RNA synthesis machinery of nonsegmented negative-strand (NNS) RNA viruses comprises a genomic RNA encapsidated with nucleocapsid (N) protein (N-RNA), associated with the RNA dependent RNA polymerase (RdRP). The RdRP is contained within a viral large (L) protein, which associates with N-RNA via a phosphoprotein (P) cofactor. Working with vesicular stomatitis virus (VSV), we developed an in vitro polymerase assay using highly purified recombinant enzyme, and a chemically synthesized naked RNA template. This in vitro RNA synthesis assay is a powerful tool to discover and study inhibitors of polymerase activity. We exploited this assay to study the effects of other viral proteins on the RNA synthesis activity by L, defining critical roles of P and N in processivity of the RdRP, and a suppressive role of the viral matrix (M) protein. We also examined the ability of different nucleotide analogs to be incorporated by polymerase in vitro and to be copied when they are present in the template. We find that Z’ substituted nucleotides have different impacts on RNA synthesis initiation and elongation, and that the sensitivity of the polymerase to such substituted nucleotides is altered by the presence of the template associated N protein. We have adapted this assay to a suite of human pathogenic viruses including measles, rabies and respiratory syncytial virus. This work provides a simple and adaptable in vitro assay to study the polymerases of negative-sense RNA viruses, defines the viral protein requirements for polymerase processivity, and inhibition, and facilitates mechanism of action studies of candidate polymerase inhibitors in vitro.
137 Optogenetic Approaches for Measuring Motor Function Deficits in Arboviral Encephalitis
John D. Morrey, Hong Wang, Venkatraman Siddharthan, Neil E. Motter, Joseph W. Clyde, Justin G. Julander
Utah State University, Logan, USA

Motor function deficits and paralysis are recognized as serious outcomes of arboviral encephalitis in human patients. We used optogenetic approaches to quantify motor deficits in the cervical and lumbosacral spinal cords of rodents infected with arboviruses, including West Nile virus. Channelrhodopsin2 (ChR2), a bacterial light-activated ion channel, was expressed in the spinal cords of hamsters from a neuron-specific synapsin promoter using adeno-associated virus 5 vector, and in transgenic mice using a motor neuron-specific choline acetyltransferase promoter. Optogenetic technology is based on the opening of the ChR2 ion channels when exposed to light, which generates action potentials in neurons, and in our studies, the subsequent contraction of muscles. Phrenic and hind limb motor functions were measured in these rodents by illuminating the spinal cords with a fiber optic laser, and measuring the electromyography (EMG) responses in the diaphragm and gastrocnemius muscles. The optogenetic responses of rodents infected with arboviral encephalitides were dramatically diminished, even to below the limits of detection in severely affected animals. This innovative optogenetic model facilitates the evaluation of potential therapies for motor function deficits. Support: NIAID U54 AI-065357, NIAID/NIH HHSN272201000039I, and Utah Agriculture Research Station UTA00424

138 Antiretroviral Therapy (ART) May Reduce the Elevated Levels of Tumor Marker in HIV/ AIDS Patients
Saif Ullah Munshi, Afsana Mitij, Nahida Sultana, S M Rashed Islam, Shahina Tabassum
1Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

BACKGROUND: Since the introduction of antiretroviral therapy (ART), among HIV/AIDS patients non-AIDS-defining malignancies (NADM) are more prevalent than AIDS-defining malignancies. Among these, gastrointestinal, lung, breast and prostate cancers are frequently seen. Several non-specific tumor markers are used for diagnosis and monitoring these tumors. However, feasibility of them as tumor markers has not been studied among the HIV/AIDS patients and patients on ART.

METHODS: Carcinoembryonic antigen (CEA), Carbohydrate antigens 19.9 (CA19.9) and Prostate-specific antigen (PSA), were tested among different groups of HIV/AIDS patients i.e. asymptomatic HIV patient [n=16, Male (M): Female (F)= 8:8], symptomatic patients [n=21, M:F= 12:9] and same symptomatic patients after ≥ 3 months of ART and patients on ART for >2 years [n=19, M:F= 12:7] by chemiluminescence. CD4/ CD8 cell & Viral Load were measured by flowcytometry and real-time PCR respectively. Student's t-tests was performed to compare the levels of biomarker between different groups and Pearson Correlation was performed to observe the association of the tumor markers with CD4 / CD8 T cell count & viral load.

RESULTS: Higher mean levels of CA19-9 (19.32 u/ml) and CEA (3.44 ng /ml) were observed in symptomatic than the asymptomatic group. Among the symptomatic patients, 5 (20.8%) and 4 (16.6%) had abnormal level of CA19-9 and CEA respectively. The mean level of CA19.9 reduced to 8.62 u/ml (p < .05) and CEA to 1.41 ng/ml (p > .05) after ART initiation and showed significant but weak negative correlation (r = -.24 to -. 30, p < .05) with CD4 / CD8 cell count. No correlation of levels of CA19-9 and CEA was observed with viral load.

CONCLUSIONS: This study revealed increasing trend of CA19.9 and CEA levels among the symptomatic HIV patients, which reduced after ART. This finding indirectly poses doubt regarding the role of ART in tumor generation. On the other hand, it could also be correct that these tumor markers may not be appropriate for screening of those tumors in HIV/AIDS patients.
Entecavir reduces Viral Load and Hepatic Injury in Chronic Hepatitis B but Fails to Normalize Immunological Changes

Saif Ullah Munshi, Nusrat Sultana, Manzurl Haque, Shahina Tabassum
Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh

The Peripheral Blood Mononuclear Cells (PBMCs) are involved in immune response. These also harbor Hepatitis B virus (HBV). Therefore effects of antivirals on PBMCs are important to study in chronic hepatitis B (CHB) infection. In this study, the expression of genes related to immune response and DNA replication i.e. CCR5, FOXP3, IP10 & MCM5 in PBMCs of patients in different stages of HBV infection i.e. acute viral hepatitis B (AVH-B, n=20), asymptomatic chronic carrier (ASC, n=20), treatment naive CHB (n=30) and 30 CHB patients who were on Entecavir (TCHB, n=30) for 1 year were studied by reverse transcription-polymerase chain reaction (RT-PCR) and compared with healthy control (HC, n=10). The expression of genes was correlated with serum HBV-DNA load and ALT levels. Comparing with the HC, all of the four genes were significantly upregulated around ~2.5 and ~2.0 fold in AVH-B and ASC patients (p< 0.01) respectively. The expression of those genes showed >2.5 fold upregulation in CHB patients (p< 0.01). The TCHB group showed < 2 fold upregulation of all the genes but the fold change was significantly lower than CHB patients (p< 0.01) and higher than HC (p< 0.01). The mean ALT level of the AVH-B patients was 276.65±25.78 (IU/L), 34.15±0.90 for ASCs, 138.37±47.67 for CHB and 40.63 ± 3.06 in TCHB patients. The mean viral load of ASCs patients was 1.53±0.25 [log10 (copies/ ml)] while in the CHB patients it was 6.65±1.06. Expression of the selected genes was positively correlated with serum ALT (r=0.74 to r=0.56) and HBV-DNA load (r=0.63 to r=0.53) in both CHB and TCHB patients. The expression of the selected genes remained upregulated in TCHB patients irrespective of the HBeAg status and viral load in comparison to HC. The present results suggest that though Entecavir reduces viral load and hepatic injury but immunological changes do not return to normal, indicating incomplete recovery from HBV infection.

P-Body Component MOV10 Inhibits HCV Virus Production and Infectivity

Tanyaradzwa Ndongwe1,2, Robert Ralston1,2,3,4, Taisuke Izumi5, Vinay Pathak5, Stefan Sarafianos1,2,6
1Christopher Bond Life Sciences Center, Columbia, USA, 2Department of Molecular Microbiology & Immunology, University of Missouri, School of Medicine, Columbia, USA, 3Liver Center, University of Kansas Medical Center, Kansas City, USA, 4Department of Pharmacology and Toxicology, Kansas City, USA, 5Viral Mutation Section, HIV Drug Resistance Program, National Cancer Institute-Frederick, Frederick, USA, 6Department of Biochemistry, University of Missouri, Columbia, USA

Mov10 is an antiviral host factor that restricts replication of retroviruses, including HIV-1. Mov10 has also been reported to inhibit hepatitis C virus (HCV). However, the mechanism of this inhibition has not been studied. Here we investigate the effect of Mov10 on HCV infection, and determine which steps of the viral lifecycle are affected by overexpression of Mov10. We demonstrate that overexpression of Mov10 in human hepatoma cells restricts HCV RNA production from a sub-genomic replicon (genotype 1a) and fully infectious virus (genotype 2a). Inhibition of RNA replication in the infectious virus system leads to decreased virus production over time, as measured by HCV RNA levels in cell culture media by qRT-PCR, and the viral titer (TCID50/ml) of released virus. In addition to decreasing virus production, overexpression of Mov10 in producer cells decreases the infectivity of the produced virus. In contrast, overexpression of a control p-body protein, Dcp1a, had no effect on HCV RNA production, virus production, or infectivity of progeny virus. The effect of Mov10 on HCV infection was not mediated by up-regulation of type I interferon. Finally, experiments with Mov10 active site mutants demonstrated that neither Mov10’s putative helicase function nor localization to p-bodies was required for antiviral activity.
141 Galectin-3 Interacts with HIV-1 Tat in Latently Infected Cells
Mika Okamoto, Akemi Hidaka, Masaaki Toyama, Masanori Baba
Kagoshima University, Kagoshima, Japan

Galectin-3, a member of the lectin family binding to β-galactoside, is widely expressed in various cells and plays an important role in cell proliferation and differentiation and modulating inflammation. We have recently demonstrated that Galectin-3 promotes HIV-1 expression in latently infected cells through NF-κB activation. HIV-1 Tat is a viral trans-activation factor essential for efficient HIV-1 transcription, and it was reported that the expression of Galectin-3 was upregulated in the cells transfected with a Tat-producing vector. In this study, we have shown for the first time that Galectin-3 directly interacts with Tat in latently infected cells. The level of Galectin-3 was found to correlate with strong expression of Tat in the latently infected cell line OM-10.1 after stimulation with TNF-α, which was determined by double immunnochemical staining with specific anti-Galectin-3 and anti-Tat antibodies. Flow cytometric analysis revealed that Galectin-3 was expressed in only Tat-expressing OM-10.1 cells irrespective of TNF-α stimulation. Furthermore, direct interaction between Galectin-3 and Tat in TNF-α-stimulated OM-10.1 cells was demonstrated by co-immunoprecipitation. We also found that the expression of Galectin-3 was significantly upregulated in PBMCs at a late stage of infection with both R5 and X4 HIV-1. These results suggest that Galectin-3 is involved in the activation of HIV-1 expression in latently infected cells through its interaction with Tat. Thus, Galectin-3 may be a potential target for inhibition of HIV-1 replication by small-molecule compounds.

142 Discovery of a Small Molecule Compound Series with Potent Activity against Hepatitis C Virus NS4B by Screening using Encoded Library Technology
Michael Thomson1, Zhengrong Zhu2, Hamilton Dickson1, Derek Parks3, Jesse Keicher1, Ken Lind2, Randy Bledsoe1, Christopher Arico-Muendel2
1AV DPU, GlaxoSmithKline, RTP, USA, 2ELT Boston, GlaxoSmithKline, Boston, USA, 3Biological Sciences, GlaxoSmithKline, RTP, USA

Encoded library technology (ELT) enables purified proteins not amenable to standard biochemical screening methods to be tested against large combinatorial libraries in a short period of time. The hepatitis C virus (HCV) NS4B protein is an integral part of the virus replication complex, but is not an obvious screening target as it does not possess robust enzymatic activity. We tested NS4B against several DNA encoded combinatorial libraries (DEL) and identified a single DEL feature that was subsequently progressed to off-DNA synthesis. The most active of the initial synthesized compounds had IC50s of 50-130 nM in a NS4B radioligand binding assay and 300-500 nM against a NS4B replicon assay. Chemical optimization yielded compounds with potencies as low as 20 nM in an HCV genotype 1b replicon assay, 500 nM against genotype 1a and 5 uM against genotype 2a. Through testing against 2a-1b chimerical replicons and other genotypes, and through resistance passage in 1b replicon, we confirmed these compounds were acting on a region of NS4B thought to comprise the first transmembrane region of the protein. A single mutation (F98L) was identified as responsible for resistance, and thought to largely explain the relative lack of potency of this series against genotype 2a. The discovery of this novel compound series highlights ELT as a valuable approach for screening against non-enzymatic drug targets.

143 Control of SAMHD1 Mediated Restriction of HIV-1 Replication
Eduardo Pauls, Roger Badia, Marc Permanyer, Eva Riveira-Muñoz, Bonaventura Clotet, Ester Ballana, Jose Este
AIDS Research Institute – IrsiCaixa, Badalona, Spain

SAMHD1 inhibits HIV-1 reverse transcription by decreasing the pool of intracellular deoxynucleotides in myeloid and lymphoid cells. The activity of the HIV-1 restriction factor SAMHD1 is controlled by cyclin-dependent kinase (CDK)-mediated phosphorylation. However, the exact mechanism of SAMHD1 regulation in primary cells is unclear. Pan CDK inhibitors roscovitine and purvalanol A show in vitro activity against several CDK, including CDK2 and AT7519 is a preferential inhibitor of CDK2. These CDK inhibitors reduced SAMHD1 phosphorylation in macrophages and CD4+ T lymphocytes as measured by Western blot analysis. HIV-1 replication was blocked in primary macrophages by roscovitine (47.3%±3.9; n=4), purvalanol A
Abstracts

144 Protective Efficacy of Novel mRNA Vaccines Against Influenza Virus Infection

B. Petsch1, M. Schnee1, A. Vogel2, D. Voss1,4, K.-J. Kallen1, L. Stitz2, T. Kramps1,3
1CureVac GmbH, Tübingen, Germany, 2Friedrich-Loeffler-Institut, Institute of Immunology, Greifswald, Germany, 3present adress: Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany, 4present adress: Roche Pharma AG, Grenzach-Wyhlen, Germany

Influenza A viruses (IAVs) have a segmented genome and effective vaccination is challenging. Especially reasortment processes and pandemic outbreaks point out the need for a new technology that allows rapid adaptation of vaccines. In this context, mRNA vaccines were designed for prophylactic vaccination against IAVs. In mice, immunogenicity and protective efficacy were analyzed. mRNA vaccines induce protective immunity, elicit B and T cell–dependent protection and target multiple antigens, including the highly conserved viral nucleoprotein, indicating its usefulness as a cross-protective vaccine. Also in larger animal models, the mRNA vaccine induced a protective immune response. Moreover, we extrapolated our knowledge to develop a mRNA vaccine against Rabies and demonstrated again protection against the viral disease. Thus, mRNA vaccines could address substantial medical need in the area of influenza prophylaxis and extend the field of anti-infective vaccinology.

145 Isolation and Characterization of a Novel Class of Potent anti-HIV Proteins from the Australian Soft Coral Synthecium sp.

Koreen Ramessar1, Chang Yun Xiong1, Lauren R.H. Krumpe2, Raymond C. Sowder II3, Robert W. Buckheit Jr.4, James B. McMahon1, Barry R. O’Keefe1
1Molecular Targets Laboratory, Center for Cancer Research, National Cancer Institute, Frederick, USA, 2Molecular targets Laboratory, Leidos Inc., Frederick, USA, 3AIDS and Cancer Virus Program, Leidos Inc., Frederick, USA, 4Imquest BioSciences Inc., Frederick, USA

An estimated 34 million people are living with the human immunodeficiency virus (HIV) worldwide. Increasing resistance to antiretrovirals (ARVs) challenges current therapies and it is critical to identify novel non-ARV anti-HIV agents to prevent infection. Here we report the isolation of a novel class of proteins, called Cnidarins, from the soft coral Synthecium sp. (phylum Cnidaria). The proteins were purified by sequential ethanol and ammonium sulphate precipitation followed by hydrophobic interaction chromatography. The purified proteins, CNID-1, CNID-2 and CNID-3, were monomers of ~170 amino acids with molecular weights of ~18 kDa. CNID-1 and CNID-3 (fully sequenced) showed no significant homology (>25%) to any known protein. All three cnidarins showed picomolar to low-nanomolar activity against laboratory strains and primary isolates of HIV-1. They inhibited viral fusion in a concentration-dependant manner but not viral attachment, indicating the proteins’ antiviral effects occur after initial virus-to-cell attachment but prior to viral entry. CNID-1 was the most potent (EC50 of 85 pM) and bound to viral glycoproteins gp120 and gp41 equally in a concentration-dependant manner, but not to other glycoproteins or soluble CD4. Pre-treatment with CNID-1 did not block sCD4 binding to gp120 and vice versa and its gp120 binding was independent of glycosylation. Recombinant production in E.coli resulted in C-terminal truncated forms of CNID-1 (14-16kDa, EC50 of 45-150nM). Ongoing work includes optimizing expression conditions to produce fully active rCNID-1.
146 Anti-HSV-2 Activity of the Glycoconjugate PG545 in a Mouse Model of Genital Herpes Infection
Joanna S. Said1, Edward Trybala1, Eva Jennische2, Stefan Lange3, Staffan Görander1, Maria Ekblad1, Jan-Äke Liljeqvist1, Tomas Bergström1
1Department of Clinical Virology, University of Gothenburg, Goteborg, Sweden, 2Department of Medical Biochemistry and Cell Biology, University of Gothenburg, Goteborg, Sweden, 3Department of Clinical Bacteriology, University of Gothenburg, Goteborg, Sweden

In search for microbicides intended for prophylaxis against infection with human immunodeficiency virus 1 (HIV-1), we have earlier shown that the glycoconjugate PG545 exhibited a virucidal potency against this virus in vitro. The compound was also effective against herpes simplex virus type 2 (HSV-2), the cause of recurrent genital herpes and a known promoter of HIV-1 transmission in humans. In this study, we investigated the antiviral effect of PG545 in a mouse model of genital HSV-2 infection with high virus inoculums (1x10^5 PFU/animal, >20 x 50% lethal dose). Preincubation of HSV-2 with PG545 at high and medium doses (10 or 2 µg/animal, respectively) completely abrogated genital infection and genital disease. At a low dose of PG545 (0.4 µg/animal), no mortality and low disease scores were recorded despite that the viral loads in vaginal washes, in dorsal root ganglia and in spinal cord including cauda equina were comparable to those of untreated controls. By immunohistochemistry, neuronal cell bodies in ganglia were stained virus-positive in low-dose treated and control mice, but ganglia remained HSV-2 antigen negative when higher concentrations of PG545 were used. Interestingly, the low-dose combination with HSV-2 and PG545 appeared to prevent viral infection of the second order of neurons located in the grey matter in the posterior, sensory, horns. However, virus-positive staining was detected in proximity to the cauda equina. By time-of-addition experiments, instillation of the compound shortly before vaginal infection significantly reduced mortality and disease score in the mouse model, while prophylaxis given at 2h before infection had no effect. No local or systemic toxicity was noted. Taken together, the PG545 might be a compound suitable for further development as a microbicide, with dual action against HSV-2 and HIV.

147 Enhancing Clinical Competency at Every Angle-ViroChannel as a New Model for Clinical Education
M Selbovitz1, D Miller1, D Lecavalier2
1Cornell ACTG, New York, USA, 2ViroChannel, Montreal, Canada

BACKGROUND: ViroChannel serves as a new model for dynamically improving clinical outcomes.

METHODS: ViroChannel provides video segments and articles on enhanced conference coverage and interviews with key opinion leaders on clinical and scientific developments.

RESULTS: ViroChannel has now secured a significant audience of clinicians in diverse settings resulting in dynamic improvements in their knowledge of clinical practices.

CONCLUSION: Data will be presented to demonstrate the value of such information to prescribers across a spectrum of viral diseases.

148 Design and Synthesis of Novel Flouroquinolones as Potential inhibitors of HIV Integrase
Periyasamy Selvam1, M Kathur Reddy2, Yves Pommier2, Christophe Marchand2
1Nova College of Pharmaceutical Education and Research, Ibrahimpatnam, Krishna Dt, India, 2Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, Maryland, USA

BACKGROUND: AIDS is a fatal pathogenic disease caused by retrovirus Human Immuno- deficiency Virus (HIV). The only available option is chemotherapy (HAART) that can reduce the viral load and improve the quality of life of HIV/AIDS patients. Present therapeutic agents are suffering with emergence of resistance, thus demanding novel targets to sustain the treatment and enhance the life span of the infected population. HIV integrase is crucial enzyme for HIV replication and fully validated therapeutic target for designing newer anti-HIV agents. Quinolone is a versatile lead molecules for designing of potential antiviral agents and flouroquinolone derivatives were reported for broad-spectrum antiviral
activity including anti-HIV activity. Present work is to study HIV integrase inhibitory activity of Novel N-Sulphonamidomethylflouroquinolone derivatives.

**METHOD:** Series of Novel N-Sulphonamidomethylflouroquinolone derivatives have been synthesized and investigated for the inhibition of HIV-1 integrase enzymatic activity. All the newly synthesized fluoroquinolones were investigated for inhibition of both 3' processing (3'P) and strand transfer process (ST) of HIV-1 integrase enzymatic activity. **RESULTS:** Compounds GF-SDM, GF-SD and NF-SD exhibited inhibitory activity against HIV-1 integrase enzyme (3'P IC₅₀: 1.5-88 µM and ST IC₅₀: 0.90-65.50 µM). GF-SD displayed significant inhibitory activity against both step of HIV Integrase enzymatic activity (3'P IC₅₀: 1.5 µM and ST IC₅₀:0.90 µM). This series Fluoroquinolones are suitable lead molecules for further molecular modifications. HIV Integrase inhibitory activity of N-Sulphonamidomethylflouroquinolone

<table>
<thead>
<tr>
<th>compounds</th>
<th>IC₅₀ a : 3-P (µM)</th>
<th>IC₅₀ STb : (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF-SDM</td>
<td>&gt;111</td>
<td>75</td>
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<tr>
<td>CF-SD</td>
<td>&gt;111</td>
<td>&gt;111</td>
</tr>
<tr>
<td>GF-SDM</td>
<td>88</td>
<td>5.6</td>
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<tr>
<td>GF-SD</td>
<td>1.5</td>
<td>0.90</td>
</tr>
<tr>
<td>GF-SA</td>
<td>&gt;111</td>
<td>&gt;111</td>
</tr>
<tr>
<td>NF-SDM</td>
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<td>NF-SD</td>
<td>10.65</td>
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</tr>
<tr>
<td>NF-SA</td>
<td>&gt;111</td>
<td>&gt;111</td>
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</tbody>
</table>

The results are IC₅₀ ±S.D, n = 2 for HIV-1 IN inhibitory activity aConcentration required to inhibits 3’ processing reaction, bConcentration required to inhibits strand transfers reaction,

149 **LEDGF- HIV Integrase Inhibitory Activity of 2-methylpyrazole and 2-amino-4-phenyl-thiazole**

Periyasamy Selvam¹, Guoping Hu², Yun Tang², Xi Li², Jin Huang²

¹Nova College of Pharmaceutical Education and Research., Jupudi, India, ²School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

Background: During the early stage of HIV-1 replication, integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Previous studies have demonstrated that HIV-1 Integrase interacts with a cellular lens epithelium-derived growth factor (LEDGF/p75) and that this viral/cellular interaction plays an important role for tethering HIV-1 pre-integration complexes (PICs) to transcriptionally active units of host chromatin. Small molecule inhibitors of HIV IN/LEDGF have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS. Present work is to investigation of 2-methylpyrazole and 2-amino-4-phenyl-thiazole as potential inhibitor of HIV Integrase/ LEDGF interaction. Method: 2-methylpyrazole and 2-amino-4-phenyl-thiazole molecule were synthesized and investigated for HIV Integrase/LEDGF interaction inhibition assay by using ALPHA screen technique and molecular modeling studies also carried by using computational methods. Results: 2-methylpyrazole and 2-amino-4-phenyl-thiazole inhibits HIV IN/LEDGFinteraction (protein-protein interaction) at the concentration of 14.03 and 12.19 µM and compound 2-amino-4-phenyl-thiazole (2A4PT) more potent compound. From molecular modelling study indicates that all the studied compounds bind with active site of HIV integrase (DDE), change the conformation and interrupt the binding of HIV integrase with LEDGF. Conclusion: 2-methylpyrazole and 2-amino-4-phenyl-thiazole are the novel class of inhibitors of HIV IN/LEDGFinteraction and this lead molecule is suitable for further modifications.
Investigation of Anti-HIV Activity and Cytotoxicity of MORINDA CITRIFOLIA L NONI Fruit Extracts

Periyasamy Selvam¹, T Paul Pandi¹, Christophe Panecouque², Erik De Clercq²
¹Nova college of Pharmaceutical Education and Research, Jupudi, India, ²Rega Institute for Medical Research, Leuven, Belgium

BACKGROUND: The development of antiviral drugs has provided crucial new means to mitigate or relieve the debilitating effects of many viral pathogens. A rich source for the discovery of new HIV infection inhibitors has been and continues to be, the ‘mining’ of the large diversity of compounds already available in nature and specifically those from botanical extracts. Morinda citrifolia is used in the Indian system of medicine for the treatment of variety of diseases including HIV/AIDS. Present work is to study anti-HIV activity and cytotoxicity of Morinda citrifolia L noni fruit extracts. METHOD: Morinda citrifolia fruit extracts were tested for inhibition of HIV-1 and -2 replication in MT-4 cells. Cytotoxicity also tested in mock-infected MT-4 by tetrazolium assay. All the extracts of Morinda citrifolia were investigated for inhibition of HIV Vpr localization and HIV Vpr induced cell apoptosis in HeLa cells to understand effect of Noni on HIV Vpr function. RESULTS: Acetone extract (MCF-AC) exhibited inhibitory activity against HIV-1 replication (IC₅₀: 157.0±37.04 µg/ml and CC₅₀: 771.67±44.69 µg/ml) in MT-4 cells and fixed oil isolated from acetone extract exhibited cytotoxicity at high concentration (771.67±44.69 µg/ml). Anti-HIV activity of acetone extract may be due to inhibition of HIV integrase enzymatic activity, since acetone extract demonstrated for potent inhibitors of HIV Integrase (Selvam et al., 24th ICAR, Antiviral research 2010). Compounds isolated from methanol and ethanol extracts of noni inhibits the HIV VPR induced cell apoptosis in HeLa Cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Strain</th>
<th>IC₅₀ (µg/ml)</th>
<th>CC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-Me</td>
<td>IIIb</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td>MCF-ET</td>
<td>IIIb</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td>MCF--AC-OIL</td>
<td>IIIb</td>
<td>&gt;534.75</td>
<td>534.75±226.36</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;534.75</td>
<td>534.75±229.36</td>
</tr>
<tr>
<td>MCF--AC-EXT</td>
<td>IIIb</td>
<td>157.0±37.04</td>
<td>771.67±44.69</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>&gt;771.67±44.69</td>
<td>771.67±44.69</td>
</tr>
<tr>
<td>Azidothymidine</td>
<td>IIIb</td>
<td>0.0015</td>
<td>&gt;25.00</td>
</tr>
<tr>
<td></td>
<td>ROD</td>
<td>0.0016</td>
<td>&gt;25.00</td>
</tr>
</tbody>
</table>

a Concentrations required to inhibit the cytopathic effect of HIV-1(IIIb) in MT-4 cells by 50%. b Concentrations required to cause cytotoxicity to 50% of the MT-4 cells whereas HIV-1 = (IIIb), HIV-2 = (ROD). All the value of SD of two independent experiments
151 **Design, Synthesis and Molecular Modelling studies of Quinazolin-3(4H)-one derivatives as novel inhibitors of HIV Integrase**

Periyasamy selvam¹, Nouri Neamati², Tino Sanchenz²

¹Nova College of Pharmaceutical Education and Research, Jupudi, Krishna Dt, India, ²Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, USA

**BACKGROUND:** HIV integrase is crucial enzyme and no human cellular counter part. HIV integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. HIV integrase is fully validated therapeutic target for designing of potential anti-HIV agents. Small molecule inhibitors of HIV Integrase have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS. Present work is to Design, Synthesis and molecular modelling studies of Quinazolin-3(4H)-one derivatives as potential inhibitors of HIV replication and HIV integrase activity. Method: Novel Quinazolin-3(4H)-one derivatives were synthesized and tested for anti-HIV activity against HIV-1 and -2 in MT-4 cells. Synthesized compounds were also investigated for inhibition of HIV integrase enzymatic activity to understand the mechanism of antiviral action. Results: Quinazolin-3(4H)-one derivatives (Q-PABA and Q-AA3) inhibits HIV Integrase enzymatic activity (Strand transfer process) with inhibitory concentration of 33 and 40 µg/ml, respectively. Conclusion: Quinazolin-3(4H)-one derivatives (Q-PABA and Q-AA3) are the novel class of inhibitors of HIV Integrase activity and Quinazolin-3(4H)-one lead molecule is suitable for further molecular modifications.

152 **Brincidofovir (BCV, CMX001) Delivers High Intracellular Concentrations of Cidofovir Diphosphate**

Phiroze Sethna, Dean Selleseth, Andrew Bae, Laurie Keilholz, Bernhard Lampert, Randall Lanier

Chimerix Inc, Durham, USA

**BACKGROUND:** Brincidofovir (BCV, CMX001) is a lipid conjugate nucleotide in Phase 3 development for the prevention of CMV infection in HCT recipients. BCV is administered orally, circulates as BCV, and is converted to the active antiviral cidofovir diphosphate (CDV-PP) within cells. BCV shares the broad-spectrum antiviral activity of CDV against all five families of dsDNA viruses which cause disease in humans. The 50 to 500-fold improved in vitro activity of BCV vs CDV has been hypothesized to result from more efficient transport of circulating BCV across the cell membrane, resulting in higher intracellular concentrations of CDV-PP. Five cell types used for determination of EC50s were exposed to identical concentrations of BCV and CDV and evaluated to determine the intracellular concentration of CDV-PP.

**METHODS:** Human foreskin fibroblast cells, MRC-5 cells, A549 cells, HepG2 cells and Vero cells were treated with 1µM of BCV or CDV for 72 hours. Cells were rinsed thoroughly to remove residual BCV or CDV and immediately extracted in methanol/water (70:30). Intracellular levels of BCV, CDV and CDV-PP were determined by LC/MS/MS.

**RESULTS:** BCV exposure resulted in 20 to 140-fold higher intracellular concentrations of CDV in the 5 cell types as compared to CDV-treated cells. The concentration of CDV-PP in BCV-treated cells was 33 to 450-fold higher than that measured in CDV-treated cells.

**CONCLUSIONS:** CDV-PP has been shown to act as an alternative substrate for viral DNA polymerases. Since the primary mechanism of viral growth inhibition involves CDV-PP as a competitive inhibitor of the natural substrate for viral polymerases, higher intracellular concentrations should be more effective and the EC50 should be lower. These data demonstrate more efficient intracellular delivery of CDV and CDV-PP by BCV versus CDV as predicted. In addition to improved efficacy, BCV may provide an improved safety profile through lower plasma concentrations of CDV, a compound noted for its renal toxicity due to preferential uptake by human organic anion transporters (hOATs) and resulting high concentration in the proximal renal tubules. The significantly improved safety profile of BCV versus CDV is partly attributable to the inability of hOATs to recognize BCV.
153 Nucleic Acid Scavengers: Impact on Inflammatory Disorders and Viral Susceptibility
Kara L. Shumansky, Eda K. Holl, Angelo Moreno, George A. Pitoc, Elizabeth Ramsburg, Bruce A. Sullenger
Duke University Medical Center, Durham, USA

The Toll-like receptor (TLR) family, TLRs 3, 7 and 9 are key components in initiation and progression of autoimmune disorders such as systemic lupus erythematosus (SLE). These TLRs are often referred to as nucleic acid-sensing TLRs due to their ability to recognize DNAs or RNAs produced by pathogens or damaged cells. During SLE progression these receptors recognize self nucleic acids/complexes, and contribute to inflammatory cytokine production and subsequent enhancement of serum autoantibody levels. We have recently discovered a new class of nucleic-acid scavenging agents that can neutralize the pro-inflammatory effects of nucleic acids on immune cells implicated in autoimmune disease development. We have shown that these nucleic acid-scavenging polymers can inhibit TLR activation and subsequent cytokine production by both dendritic cells and B cells of wild type and lupus prone mice. Moreover, stimulation of immune cells by encapsulated viral particles is unaffected in the presence of polymers. These findings are further supported by an in vivo model of murine influenza. Infected animals treated with polymer undergo a normal disease progression, and their immune response to said infection remains fully intact. Thus showing that the effects observed in the initiation and maintenance of the immune response in the presence of polymers is specific to nucleic acid stimulation and not overall immune suppression. Finally, we have shown that nucleic-acid binding polymers can prevent skin lesions following mechanical injury in lupus prone animal models. These findings provide a new avenue in drug development as these agents can potentially be utilized to block overt autoimmune disorders while allowing normal immune responses to occur.

154 Targeting an Immunomodulatory West Nile Virus Protein to Improve Vaccine Candidate Efficiency
Lindsey Stevenson, Frank Scholle
North Carolina State University, Raleigh, USA

Toll-like receptor (TLR) 3 is expressed on endosomal membranes of many cell types. TLR3 recognizes double stranded RNA (dsRNA), an intermediate of West Nile Virus (WNV) replication, initiating production of Type I interferon and pro-inflammatory cytokines by infected cells. These cytokines contribute to activation of naïve T and B cells and induction of protective memory. WNV’s genome codes for several multifunctional proteins which primarily function in viral replication, but can also have immunomodulatory properties. One such protein is non-structural protein 1 (NS1), which is required for viral genome replication, and which we have shown to be able to inhibit TLR3 signaling both in vitro and in vivo. NS1 is secreted to high levels from infected cells, and can bind back to both infected and uninfected neighboring cells. Infection with RepliVAX, a single-cycle infection WNV vaccine candidate, has been shown to protect mice against subsequent lethal WNV infection. However, RepliVAX infected cells also secrete NS1. The TLR3 inhibiting effects of NS1 could reduce RepliVAX’ immune stimulating properties, and therefore its effectiveness as a vaccine. We have generated a mutant RepliVAX whose NS1 still supports viral replication, but does not inhibit TLR3 signaling. We hypothesize that fewer viral particles of this RepliVAX mutant will be required to stimulate a protective response in mice compared with wild-type RepliVAX, resulting in a better vaccine candidate.
155 **Effect of a Triple Anti-Enteroviral Combination Applied in Consecutive Alternative Administration (CAA) Course in Coxsackievirus B1 Neuroinfection in Mice**

**A. Stoyanova, I. Nikolova, A. S. Galabov**

*The Stephan Angeloff Institute of Microbiology, Bulg. Acad.Sci., Sofia, Bulgaria*

Human enteroviruses (EV) distributed worldwide are causative agents of a broad spectrum of diseases with high morbidity of CNS, heart, endocrine pancreas, etc., and of common cold, as well. This formulates chemotherapy as the main tool for control of EV infections. At present, clinically effective anti-EV drugs do not exist. The main reason for this is the development of drug resistance. The monotherapy courses were the only approach used till now. For the first time in the antiviral investigations our team introduced the testing of combination effect of selective inhibitors of EV replication with different mode of action. In previous studies, we have proved the efficacy of the consecutive alternative administration (CAA) treatment course of the triple combination of disoxaril/guanidine/oxoglaucine (DGO) in CVB1 infection in newborn mice. Drug sensitivity studies of viral brain isolates from mice, treated with DGO showed not only preserved, but even increased sensitivity to the drugs included in the combination*. We have studied the effect in newborn mice infected s.c. with 20 LD50 of CVB1 of CAA treatment course with the combination pleconaril/guanidine/oxoglaucine (PGO), in which disoxaril of DGO was replaced by pleconaril. The results obtained manifested an efficacy of PGO combination administered according to the CAA course schedule in CVB1 neuroinfection in mice. It was found that the monotherapeutic course with pleconaril (oral daily doses 25-200 mg/kg), in distinction of disoxaril, was markedly effective, but this activity was accompanied by a markedly suppressive effect on the animal growth. This toxic effect of pleconaril was absent when the compound was included in PGO combination following the CAA course. The simultaneous every day administration of the compounds in PGO in analogy to DGO was without effect. Pleconaril applied individually once each three days, and the partners in the PGO, oxoglaucine and guanidine, applied every day separately were without effect, too. These data demonstrate the high efficacy of the CAA course approach and its perspectives for establishment of anti-enterovirus chemotherapy. *Vassileva-Pencheva, R., Galabov, A. S. Antivir. Res. 85, 2010, 366.*

156 **Abalone Hemocyanin Inhibits Herpes Simplex Virus Type 1 Infection in vitro**

**Negar Talaei Zanjani1, Monica Miranda Saksena1,2, Anthony L. Cunningham1,2, Peter Valtchev1, Vincent G. Gomes1, Fariba Dehghani1**

1School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, Australia, 2Centre for Virus Research, Westmead Millennium Institute, Sydney, Australia

Here, we describe the extraction of an antiviral compound from the sera of abalone Haliotis rubra, called hemocyanin and its activity against herpes simplex virus type 1 (HSV-1). Hemocyanin is a huge glycoprotein, which circulates oxygen in invertebrates such as molluscs and arthropods. A combination of plaque assay and immunofluorescence microscopy was used to understand the mode of action of this glycoprotein. This glycoprotein was extracted from sera and then purified using filters for which the molecular weight cut off was 80 times smaller than the size of the protein. In-vitro tests showed that the compound inhibited HSV-1 infection of Vero cells in a dose dependent manner with 50% effective dose (ED50) of 0.36±0.01 mg/mL and with insignificant toxicity. Our results showed that hemocyanin was present in the cytoplasm as well as on the cell surface. Our findings suggest that this glycoprotein can interfere with virus binding and/or entry into Vero and HeLa cells by direct interaction with the virus. In conclusion, our findings show that hemocyanin has a great potential as a new therapeutic approach against HSV-1.
157 Anti-influenza and Anti-inflammatory Activity of KPT-335, a Selective Inhibitor of Nuclear Export (SINE), in Mice and Ferrets

Sharon Tamir1, Olivia Perwitasari1, Scott K. Johnson1, Yosef Landesman2, Joel Ellis2, Sharon Shacham2, Robert O. Carlson2, Ralph A. Tripp1

1Department of Infectious Diseases, Animal Health Research Center, University of Georgia, Athens, USA, 2Karyopharm Therapeutics, Natick, USA

The ongoing emergence of novel and drug resistant influenza strains with pandemic potential continues to fuel a need for new anti-influenza treatments. Host targets offer the potential advantages of broad-spectrum activity across viral strains and lesser tendency for development of resistance. Exportin 1 (XPO1), which regulates nuclear export of >200 proteins, including some viral proteins, has surfaced recently as an attractive anti-influenza host target. XPO1 is essential for the influenza life cycle through regulation of viral ribonucleoprotein (vRNP) nuclear export. We have developed potent, small molecule inhibitors of XPO1, termed Selective Inhibitors of Nuclear Export (SINE), with drugs from this class proving to be well tolerated and active in the clinic for dog and human cancers. We have previously reported that the orally bioavailable SINE KPT-335 inhibited nuclear export of influenza vRNP, leading to suppression of in vitro and in vivo replication of both A and B influenza strains. We have since found potent KPT-335 activity across a broader panel of strains, including avian influenza H5N1 and H7N9. Orally administered KPT-335 showed strong activity in a therapeutic regimen against H1N1 influenza strain (A/California/04/09) in ferrets, inducing 1000-fold reduction in lung viral titer after treatment with 10 mg/kg BID for three days post infection. In mice infected with the same H1N1 strain, two doses of 20 mg/kg KPT-335 QD or QOD post infection induced over 6-fold reduction in lung viral titer. KPT-335 also induced decreased expression of the pro-inflammatory cytokines IFN-γ, IL-1β, IL-6 and TNF-α in H1N1-infected mouse lungs. Finally, toxicological studies in rats and monkeys have been initiated in preparation for clinical development. Based upon the anti-influenza activity of KPT-335 in animal models at doses that are well tolerated for a closely related SINE in humans, we believe KPT-335 has potential as an efficacious therapy for influenza.

158 Pharmacokinetics and Pharmacodynamics of Tenofovir and Tenofovir Disoproxil Fumarate in the Female Genital Tract

Ekaterina S. Taneva1, Leslie A. Geer2, Pedro M.M. Mesquita1, Betsy C. Herold1

1Albert Einstein College of Medicine, Bronx, USA, 2Particle Sciences, Bethlehem, USA

BACKGROUND: Tenofovir (TFV) and tenofovir disoproxil fumarate (TDF) are being evaluated for PrEP against HIV and HSV-2. Their efficacy depends on transport, metabolism and competition with intracellular nucleotides. However, the mechanisms of TDF and TFV entry into target cells are unknown and the biological factors modulating pharmacokinetics and pharmacodynamics (PK/PD) within the female genital tract (FGT) have not been investigated. The purpose of this study is to characterize drug uptake and to determine how alterations in the FGT mucosal environment impact PK/PD.

METHODS: Drug transport was assessed using human vaginal epithelial cells (VK2), T cells (Jurkat), and PBMCs. Intracellular drug accumulation was measured by liquid scintillation counting after exposure to radioactive drugs. Drug levels were quantified by HPLC-MS/MS. Expression of drug transporters was assessed by RT-PCR. TZM-bl cells were used to assess the antiviral activity of TFV in the presence of cervicovaginal (CVL) secretions.

RESULTS: TDF uptake was rapid and its hydrolyzed metabolites mPTFV and TFV were detected within 15 min. [3H]TDF accumulation was temperature-dependent and subject to 50% inhibition by 74-fold excess of unlabeled TDF or adefovir dipivoxil, reflecting competition for entry or esterase-mediated cleavage. In contrast, [3H]TFV uptake was slower and unaffected by excess of unlabeled TFV. [3H]TFV transport was independent of the presence of Na+ and unaffected by substrates and inhibitors of nucleoside transporters. OAT1 and OAT3, which are implicated in renal TFV uptake, were not expressed in any tested cells. However, OAT4 and OAT6 were expressed. The anti-HIV activity of TFV was modulated in the presence of CVL compared to control buffer.

CONCLUSION: Collectively, these findings indicate that TFV uptake in the FGT is not mediated by OAT1/3 and may occur by a different mechanism than reported for kidney epithelium. In addition, there is an active component to TDF intracellular accumulation either at the site of its transport or hydrolysis. The PK/PD of both drugs may be modulated by alterations in the FGT mucosal environment, thus contributing to variability in drug efficacy.
159 Weight Loss Cutoff for Mortality in Mice Impacts the Results from Combination Drug Therapy for an Influenza A (H1N1) Virus Infection

Bart Tarbet, Deanna Larson, Min-Hui Wong, Donald Smee
Utah State University, Logan, USA

Combination therapy with oseltamivir and peramivir for an influenza virus infection was used as a model to evaluate the effects of different weight loss cutoffs for mortality in mice. Because the drugs have similar modes of action, combination therapy could have additive or antagonistic effects. In vitro studies evaluating antiviral activity showed that low-dose combinations (0.032-3.2 nM oseltamivir plus 0.0032-0.1 nM peramivir) caused greater virus inhibition than either compound alone. Higher concentrations did not show this effect. A three-dimensional MacSynergy plot of the data had an overall synergy volume of 540 (high synergy), whereas the overall volume of antagonism was -44 (moderate antagonism) for a net effect of 496 (high synergy). Mice infected with Influenza A/CA/04/2009 (pandemic H1N1) virus were treated b.i.d. for 5d starting 4h post-virus challenge with oseltamivir (p.o.) or with peramivir (i.m.) as monotherapy, or in combination. The greatest effects were observed for oseltamivir at the 0.3 mg/kg dose. At this dose an increase in lifespan was observed for all combinations with peramivir. A MacSynergy plot of the data showed doses of synergy (0.3 mg/kg oseltamivir plus 0.03-0.3 mg/kg peramivir) and antagonism (1-3 mg/kg oseltamivir plus 0.3-1 mg/kg peramivir). Volumes of synergy and antagonism were 318 and -180, respectively, for a net synergy of 134 (high synergy). These results were based on a 30% weight loss cutoff for mortality. In addition, mean day of death values and MacSynergy plots were determined at weight loss cutoffs of 20%, 25%, and true mortality. Mean day of death for placebos were 4.5, 5.7, 7.3, and 9.3 days post-infection, and MacSynergy plots had net volumes for synergy of 0, 83, 138, and -40, for endpoints of 20%, 25%, 30% and true mortality, respectively. The variability in survival, based on different weight loss cutoffs for mortality, also shifted the MacSynergy results in one direction or another for low dose drug combinations. Therefore, these data show that the weight loss cutoff for mortality in mice can directly impact the results following combination drug therapy. [Partially supported by contract N01-AI-30063 (awarded to Southern Research Institute) from the Virology Branch, NIH]

160 Potential of Novel Acylguanidine-based Small Molecules with Broad-spectrum Viroporin Activity as Dual Inhibitors of HIV-1 and Hepatitis C Virus

Ian Tietjen1, Philip Mwimanzi2, Scott C Miller1, Aniqa Shahid2, Zabrina L Brumme2, Mark A Brockman2, David Fedida1
University of British Columbia, Vancouver, Canada, Simon Fraser University, Burnaby, Canada

Viroporins are virus-encoded ion channels essential for viral replication, making them attractive targets for new therapeutic agents. While recent therapeutic advances for Hepatitis C (HCV) and HIV-1 are encouraging, effective treatments for those with HIV+HCV co-infection are lacking. Here we describe a novel acylguanidine-based small molecule with broad spectrum activity against viroporins. To confirm viroporin function, we first developed a mammalian cell-based assay using a pH-sensitive fluorescent dye that localizes to intracellular vesicles. Consistent with known viroporin-regulated proton transport and vesicle alkalization, transient expression of the p7 viroporin of HCV (genotype 1b) quenched fluorescence by 38.7% vs. mock-transfected cells (p< 0.05). Moreover, p7-expressing cells treated with the known viroporin inhibitor rimantadine at 1, 3, and 10µM respectively restored fluorescence by 49.2, 57.8, and 100.7% (p< 0.05). We next used this assay to screen a novel acylguanidine-based small molecule library and found that SM111 restores fluorescence in cells expressing viroporins from HCV, BVDV, and Dengue; for example, 10µM SM111 restored fluorescence in HCV p7-expressing cells by 81.2% (p< 0.05). We then assessed the anti-HIV activity of SM111 using a multi-cycle in vitro replication assay and observed that SM111 inhibits HIV-1 strain NL4-3 by >95% at 100µM with minimal cellular toxicity. Notably, SM111 also equally inhibits replication of recombinant HIV strains encoding patient-derived HIV polymerase sequences that confer major resistance to nucleoside and non-nucleoside reverse transcriptase inhibitors, supporting a mechanism of action distinct from available antiretroviral agents. Our data suggest that SM111 and related compounds are promising prototypes for developing broad-spectrum viroporin inhibitors and antivirals for diseases like HIV+HCV co-infection.
161 **Characterization of a Novel Respiratory Syncytial Virus Inhibitor**

Choi-Lai Tiong-Yip, Lisa Aschenbrenner, Kenneth D. Johnson, Robert E. McLaughlin, Jun Fan, SreeRupa Challa, Hui Xiong, Qin Yu

AstraZeneca Infection iMed, Boston, USA

The respiratory syncytial virus (RSV) L protein is a viral RNA dependent RNA polymerase that contains multiple enzyme activities required for RSV replication. The RSV L inhibitors described in literature are limited by their cytotoxicity or the lack of RSV B subtype coverage. Here we characterize a novel RSV L inhibitor with high potency against both RSV A and B subtypes and no detectable cytotoxicity. This compound, AZ-27, was equally active against RSV live viruses and subgenomic replicons, and demonstrated advantage over other classes of RSV inhibitors in time-of-addition and cell-line dependency studies. Resistance studies identified a dominant mutation in the putative capping enzyme domain of L protein, which conferred strong resistance to the AZ-27 series but not other classes of RSV inhibitors, supporting RSV L protein as the direct target for AZ-27. This novel and broad-spectrum RSV L polymerase inhibitor has paved the way towards an efficacious RSV therapeutic and provided a new tool for interrogation of the L protein function.

162 **NGS Nominated Genes for Predisposition to Balkan Endemic Nephropathy (BEN)**


Department of Medical Genetics, Medical University, Sofia, Genomics Laboratory, Malinov Clinic, Sofia, National Center of Public Health & Analyses, Sofia, Vratza District Hospital, Vratza, Bulgaria, Faculty of Medicine, University of Nis, Serbia, University of Skopje, Macedonian Academy of Science and Art, Republic of Macedonia, Institute of Microbiology, Bulg.Acad. Sci., Sofia, Institute of Anatomy, Bern University, Switzerland

BACKGROUND: BEN is a familial chronic tubulointerstitial disease with slow progression to terminal renal failure. There are several hypotheses attempting to explain the environmental cause of this disease: aristolochic acid, ochratoxin A, viruses etc. The results of molecular investigations propose that BEN is a multifactorial disease.

METHODS: Exome sequencing of 20,000 genes with Illumina Nextera Exome Enrichment kit was performed on 22 DNA samples (11 Bulgarian patients and 11 Serbian patients). The first step of analysis included mutations with significantly different allele frequency in comparison with European populations were selected. In the second analysis we focused on non-annotated missense variants and nonsense/frameshift variants. We selected the variants with frequency of more than 40% in both patients’ groups.

RESULTS: We found two annotated genes - MTUS2 (rs928661) and ZNF534 (rs1366258) with statistically significant differences of minor alleles between BEN patients and European population (p=0.0037 p=0.0001 respectively). From non-annotated variants with high frequency, we nominated 6 genes with missense deleterious/damaging variants (AGAP4, HSPG2, USP17L2, KCNK5, ZNF367 and FRG1) and 7 genes with nonsense/frameshift variants (AOAH, IL32, CELA1, ZNF626, MLL3, FRG2C and EI24).  

CONCLUSION: Mutant genes in BEN patients belong to six functional groups – Basement membrane/ECM/cytoskeleton, Transcription/methylation, Immunity, Apoptosis/cell cycle and Development. We suggest they play role in the molecular pathogenesis of BEN.
163 Identification of novel inhibitors of HBV Replication by In Silico Screening Targeting Capsid Assembly
Masaaki Toyama¹, Takayuki Hamasaki¹, Mika Okamoto¹, Koichi Watashi², Takaji Wakita², Masanori Baba¹
¹Kagoshima University, Kagoshima, Japan, ²National Institute of Infectious Diseases, Shinjuku-ku, Japan

Hepatitis B virus (HBV) is a major cause of serious liver diseases, such as cirrhosis and hepatocellular carcinoma. Chronic HBV infection is currently treated with nucleoside analogs, including lamivudine, entecavir, and tenofovir. They are potent inhibitors of HBV DNA polymerase, which also functions as reverse transcriptase. Although these compounds are effective in the treatment of infected patients, emergence of drug-resistant mutants and viral reactivation after treatment interruption are still major concerns in antiviral chemotherapy against HBV. Therefore, it seems still mandatory to identify and develop novel inhibitors that target viral gene products other than DNA polymerase. HBV capsid assembly is a critical step in viral replication and an attractive target for inhibition of HBV. To find novel inhibitors of HBV, we constructed an in silico screening system based on X-ray crystallography of HBV core protein and examined 170,000 compounds for their interaction with the core protein in this system. Fifty compounds with high docking scores were selected, and 24 of the 50 compounds were obtained and examined for their inhibitory effect on HBV replication in HepG2.2.15.7 cells, a HepG2.2.15 clone producing a higher amount of HBV. Consequently, two different classes of compounds, 1-(diphenylmethyl)-4-methylpiperazine (cyclizine) and 7-chloro-1-(2-phenoxyethyl)-3-[[4-(pyridin-2-yl)piperazin-1-yl]methyl]quinolin-2-one, were found to be selective inhibitors of HBV replication. Although their exact mechanism of action remains to be elucidated, the two compounds are considered to be promising leads of novel HBV inhibitors. Further studies are in progress to optimize their chemical structures.

164 GPC-N114: A Novel Non-Nucleoside Inhibitor of Picornavirus RNA-dependent RNA Polymerase
Lonneke van der Linden¹,², Laia Vives-Adrián³, Barbara Selisko⁴, Bruno Coutard⁴, Gerhard Puerstinger⁵, Nuria Verdaguer³, Johan Neyts², Frank van Kuppeveld⁶
¹Dept Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, ²Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium, ³Institut de Biologia Molecular de Barcelona (CSIC), Barcelona, Spain, ⁴Laboratoire d’Architecture et Fonction des Macromolécules Biologiques, CNRS, University Marseille, Marseille, France, ⁵Dept Pharmaceutical Chemistry, Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria, ⁶Dept Infectious Diseases and Immunology, Utrecht University, Utrecht, Netherlands

The genus Enterovirus of the family Picornaviridae contains many important human pathogens such as poliovirus, coxsackievirus, rhinovirus, and enterovirus 71. Currently, there are no antiviral drugs available for the treatment or prevention of infections with these viruses. Here, we report a small molecule, GPCN114 (2,2&apos;-[(4-chloro-1,2-phenylene)bis(oxy)]bis(5-nitrobenzonitrile)), that exerts broad-spectrum anti-enterovirus activity. GPC-N114 also inhibited the replication of viruses of the genus Cardiovirus, but not of the genus Aphthovirus. Using subgenomic replicons, we show that GPC-N114 exerted its inhibitory activity at the RNA replication stage. Coxackievirus B3 (CVB3), an enterovirus, has a high genetic barrier to resistance against GPC-N114, whereas EMCV, a cardiovirus, rapidly acquired resistance due to mutations in the viral RNA-dependent RNA polymerase 3Dpol (M300V and I304V). In vitro polymerase assays demonstrated that GPC-N114 inhibited the elongation activity of both CVB3 and EMCV 3Dpol, and that mutations M300V and I304V reduced the susceptibility of EMCV 3Dpol to the inhibitor. Furthermore these assays showed that GPC-N114 inhibited polymerase activity in a competitive manner with respect to the RNA template-primer duplex. Analysis of co-crystals of the polymerase and GPC-N114 revealed that the binding site of the compound overlapped with that of the template, providing a rationale for the competitive mode of inhibition. This study presents the first picornavirus inhibitor that acts by competing with the RNA template-primer and identifies a new pocket that can be used for the design of broad-spectrum inhibitors of picornavirus replication.
165 Discovery of a Small Molecule Triggering Innate Immunity
Shihyun You, Anna Banka, Hamilton Dickerson, Margaret Gartland, Cindy Richards, J. Brad Shotwell, Michael Thomson, Mi Xie
GlaxoSmithKline, RTP, USA

The standard of care for hepatitis C virus (HCV) infected patients requires lengthy treatment with a combination of pegylated interferon α and ribavirin. Recently, the addition of direct acting antivirals including one of two recently approved NS3 protease inhibitors has dramatically improved patient response and cure rates. Ideally, an all-oral combination therapy including novel classes of HCV inhibitors will be identified. A 2.1M compound high-throughput screen was conducted using a HCV subgenomic replicon harboring the HCV replication machinery. After triaging the hits in numerous cell based as well as target specific biochemical assays, we identified GSK899 as an inhibitor with anti-HCV activity at sub uM EC50 against genotype 1a, 1b, and 2a HCV strains, but without inhibiting any specific viral enzymatic activities. An attempt to select resistant mutations failed, implying that GSK899 had a high genetic barrier to resistance. We speculated that GSK899 might target host machinery involved in either the host innate immune response or other host proteins critical for HCV replication. Treatment of HUH-7 cells with GSK899 resulted in induction of numerous interferon stimulated genes (ISGs), which are key players in the antiviral state. The levels of ISG induction were similar to that of type I interferon (IFN). Interestingly, the treatment of GSK899 did not induce the expression of IFN genes, suggesting that GSK899 mimics IFN and activates the IFN-mediated JAK-STAT pathway. The activation of the JAK-STAT pathway was confirmed by monitoring phosphorylated STAT1 upon GSK899 treatment for 10 minutes. From siRNA knock-down and overexpression experiments, JAK1 appeared to be a molecular target of GSK899, however, we have yet to demonstrate a physical interaction of GSK899 and JAK1. The activation of the JAK-STAT pathway by GSK899 was also confirmed in mice as monitored by phosphorylated STAT1 and ISG induction in the liver. Unexpectedly, GSK899 treatment also induced a significant level of pro-inflammatory cytokines such as IL6 and was poorly tolerated in mice. Here, we report a novel small molecule that mimics type I IFN and acts as a JAK1 agonist activating the JAK/STAT pathway and inducing antiviral effector genes.

166 Regulation and Function of Efflux Transporters in Mouse Cervicovaginal Tissues
Tian Zhou1,2, Minlu Hu1,2, Andrew Pearlman2, Lisa Rohan1,2
1Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, USA, 2Magee-Womens Research Institute, Pittsburgh, USA

Drug transporters play an important role in antiretroviral drug pharmacokinetics (PK) for the pre-exposure prophylaxis (PrEP) of HIV-1 sexual transmission. Emerging evidence has shown the expression of efflux transporters including P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance protein 4 (MRP4), in human cervicovaginal tissues. However, the regulation of these cervicovaginal transporters by microbicide relevant factors (e.g. endogenous and exogenous hormones), as well as their function in antiretroviral drug PK remain unknown. This study aims to examine the effect of menstrual cycle and exogenous hormones on the expression and localization of the three cervicovaginal transporters, and examine the function of cervicovaginal MRP4 in the PK of tenofovir (TFV), in a mouse model previously utilized for microbicide safety testing. The three transporters were expressed at moderate to high levels in mouse cervicovaginal tissues compared to liver. They were localized in epithelial and/or vascular endothelial cells, which are cell types relevant to drug penetration and/or elimination. The transporter mRNA level and protein localization underwent cyclic changes, as observed in mice with natural estrous cycle and those synchronized to estrus or diestrus stages by exogenous hormones. The effect by estrous cycle/synchronization depended on the type of specific tissue and transporter examined. In the mice receiving IP dosing of TFV, the co-administration of MK571 markedly increased the TFV concentrations in the cervicovaginal lavage (CVL) and vagina by several fold. Similarly, the co-administration of MK571 significantly increased the TFV concentrations in mouse endocervix and vagina after vaginal dosing of TFV gel. In conclusion, the three transporters were positively expressed and localized in mouse cervicovaginal tissues. Varying hormone levels caused by the estrous cycle or exogenous hormones affected the expression and localization of these transporters. In this model, it was found that the MRP4 transporter impacted cervicovaginal tissue distribution of vaginally and systemically administered TFV.
172 Preclinical Studies of SB 9200 as an Antiviral Agent Against HBV and HCV
R.P. Iyer1, B.E. Korba2, R.K. Pandey1, S. Padmanabhan1, J.K. Marquis1, J.M. Skell1, M.L. Harter3
1Spring Bank Pharmaceuticals, Milford, USA, 2Georgetown University Medical Center, Washington, USA, 3MPI Research, Mattawan, USA

BACKGROUND: SB 9200 is a novel nucleotide compound with potent antiviral activity against wild type and resistant variants of HBV, pan genotypic activity against HCV with EC50 25 to 180 nM, RSV (EC50, 250 nM), and Norovirus (EC50, 2 uM, EC90, 10 uM). The compound is synergistic with different classes of antiviral drugs. SB 9200 was found to activate RIG-I and NOD2, the cellular viral sensors that trigger Interferon production and induction of antiviral state in cells. For further development of SB 9200, studies were undertaken to elucidate the MOA, and to perform IND-enabling toxicology and safety pharmacology studies.

METHODS: MOA studies were done using HLE and HEK cells transfected with RIG-I and NOD2 plasmids, along with IRF3 luciferase construct as a reporter gene. Following treatment with compounds, cells were harvested and Luciferase activity measured using Dual-Luciferase Reporter Assays. IFN production was quantitated using ELISA. Dose range-finding and 14-day repeat-dose toxicity studies were conducted in rats at doses up to 500 mg/kg/day and in cynomolgus monkeys up to 360 mg/kg/day of SB 9200 administered orally. Other studies included the cardiovascular safety evaluation of SB 9200 - in vitro with hERG transfected HEK cells, and in vivo in monkeys --, as well as, respiratory and neurobehavioral studies in rats. Results: MOA studies showed that SB 9200 activated RIG-I and NOD2 resulting in 15-20 fold induction of IRF3 and enhanced expression of IFN. In the hERG assay, SB 9200 had an IC50 >200 uM. In the 14-day repeat dose toxicity study in rats, there were no significant toxicological findings and in monkeys, SB 9200 was well tolerated and toxicological findings were limited to mild and reversible increases in ALT and triglycerides with no corresponding histopathology at 360 mg/kg/day. No cardiovascular, respiratory, or neurobehavioral safety issues were identified in rats at doses up to 500 mg/kg/day.

CONCLUSION: SB 9200 is a novel, first-in-class antiviral agent with an excellent safety profile and is currently under evaluation in human clinical trials against HCV.

173 Prophylaxis with the Viral Polymerase Inhibitor 2’-C-methylcytidine Successfully Prevents Transmission of Murine Norovirus from Infected to Uninfected Mice
Joana Rocha-Pereira, Dirk Jochmans, Johan Neyts
KU Leuven - University of Leuven, Rega Institute for Medical Research, Leuven, Belgium

Norovirus outbreaks are highly prevalent, extensive and can disturb the functioning of health institutions, leading to closure of hospital wards and causing life-threatening infections in long-term care facilities. Prolonged and severe disease is less frequent but still relevant among more susceptible groups (elderly, immunocompromised, young children). To add to this large burden of disease, noroviruses are responsible for significant mortality among children in developing countries. It is therefore a pressing matter to develop strategies for the treatment and/ or prophylaxis of norovirus infections. We recently reported that the viral polymerase inhibitor 2’-C-methylcytidine (2CMC) efficiently protects against murine norovirus (MNV)-induced diarrhea and mortality in AG129 mice (Rocha-Pereira et al., 2013, J Virol 87:11798-805). We now report oral inoculation of AG129 mice (deficient in alpha/beta and gamma interferon receptors) with as low as 600 CCID50 of MNV is sufficient to result in virus-induced disease; which indicates that animals had not
174 Combination Therapy of Vaccinia Virus Infections in Immunosuppressed Mice Using Vaccinia Immune Globulin, Parenteral Cidofovir, and Topical Cidofovir

Donald F. Smee, Ashley Dagley, Brittney Downs, Brett L. Hurst
Utah State University, Logan, USA

Vaccinia immune globulin (VIG) and cidofovir (CDV) have been used to treat complications associated with smallpox (live vaccinia virus) vaccinations in humans, and often the substances were used in combination. Because severe vaccinia infections occur in immunocompromised individuals, the infections have been very difficult to treat. The amount of VIG required to treat a single individual has been enormous, considering the limited stockpile of the material available. In 2012 the U.S. Centers for Disease Control and Prevention held a workshop to discuss animal models that could be used for studying drug combination regimens that might predict how to reduce the amount of VIG used for human treatment. The present research was designed with this purpose in mind. Cutaneous vaccinia lesions were made on the backs of SKH-1 hairless mice that were treated every four days with cyclophosphamide to keep the animals immunosuppressed. Treatment with intraperitoneally (i.p.) administered VIG (5 mg/mouse, given every four days) alone or combined with either i.p. CDV (50 mg/kg/day, given every three days), topically (Top) administered CDV (0.5% cream, applied to primary lesions every three days), or triple combination (VIG + i.p. CDV + Top CDV). All treatments began 2 days after cutaneous infection, with treatments ending on day 11. Mean survival times, primary lesion sizes, and viral titers were assessed. A separate study involved using a recently developed SCID hairless mouse (SHO strain, Charles River Labs). In SHO mice, comparisons were made of the effects of double combinations (i.p. CDV + Top CDV) compared to the triple combination containing VIG. The results of the experiments clearly demonstrated that the triple combination was superior to three different double combinations (VIG + i.p. CDV, VIG + Top CDV, and i.p. CDV + Top CDV). Thus, for maximum benefit (which may translate into sparing the amount of VIG used in humans), a triple combination regimen of VIG plus parenteral (or oral, where appropriate) antiviral drug plus topical antiviral drug appears to be optimal. Supported in part by Contract No. HHSN272201000039I from the Virology Branch, DMID, NIAID, NIH.

176 A Mouse Model for a Bat Coronavirus HKU5 Variant, a Subgroup 2c Beta Coronavirus.
Sudhakar Agnihothram,1 Boyd Yount,1 Eric Donaldson,1 Andrew Mesecar,2 Arun Gosh,3 Mark Denison,3 Mark Heise1, Ralph Baric1

1Departments of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, USA, 2Department of Medicinal Chemistry, Purdue University, West Lafayette, USA, 3Departments of Pediatrics and Pathology and Microbiology and Immunology, Vanderbilt University, Nashville, USA

Cross-species transmission of zoonotic coronaviruses (CoVs) can result in pandemic disease outbreaks. MERS-CoV, identified in 2012, has caused 182 cases to date with ~43% mortality, and no small animal model has been reported. MERS-CoV shares >65% identity at the amino acid level with b-CoV subgroup 2c Pipertilus BtCoV HKU5 in several regions, including the nsp5 and N protein, which are significant drug and vaccine targets. BtCoV HKU5 has been described in silico but has not been shown to replicate in culture, thus hampering drug and vaccine studies against subgroup 2c b-CoVs. We report the synthetic reconstruction and testing of BtCoV HKU5 containing the SARS-CoV S glycoprotein ectodomain (BtCoV HKU5-SE). This virus replicates efficiently in cell culture and in young and aged mice, where virus targets airway and alveolar epithelial cells. Unlike some subgroup 2b SARS-CoV vaccines that elicit a strong eosinophilia following challenge, we demonstrate that BtCoV HKU5 and MERS-CoV N-based Venezuelan Equine Encephalitis Replicon (VRP) vaccines do not cause extensive eosinophilia following BtCoV HKU5-SE challenge. Passage of BtCoV HKU5-SE in young mice resulted in enhanced virulence, causing 20% weight loss in aged mice with diffuse alveolar damage characterized by hyaline membrane formation, and mutations in nsp13, nsp 14, and ORF5 and M genes. Finally, we identify an inhibitor active against the nsp5 proteases of subgroup 2c b-CoVs. Synthetic genome platforms capable of reconstituting emerging zoonotic viral pathogens or their phylogenetic relatives provide new strategies for identifying broad-based therapeutics, evaluating vaccine outcomes, and studying viral pathogenesis.
177 AVI-7288 Provides Significant Survival Benefit for Marburg Virus Infection
Patrick L Iversen1,2, Alison Heald1, Travis K Warren3, Jay Charleston1, Peter Sazani1, Amy C Shurtleff3, Lisa Welch3, Sina Bavari3
1Sarepta Therapeutics, Cambridge, USA, 2Oregon State University, Corvallis, USA, 3USAMRIID, Fredrick, USA

Objectives: Marburg virus (MARV) is a highly virulent RNA virus of the family Filoviridae and a causative agent of viral hemorrhagic fever (VHF) in humans. In the cynomolgus macaque VHF model, death occurs 7 to 12 days post-infection. The objective was to evaluate the therapeutic benefit provided by AVI-7288 targeting the nucleoprotein (NP) gene and assess its safety. Methods: Five groups of six cynomolgus macaques per group (both male and female) were infected by subcutaneous injection with a target dose of 1,000 plaque forming units of MARV Musoke at time zero. AVI-7288 was administered at 15 mg/kg intravenously (IV) once a day for 14 days with the first dose delivered in group 1 at 1 h, in group 2 at 24 h, in group 3 at 48 h, and in group 4 at 96 h post-infection. Group 5 received phosphate buffered saline vehicle IV daily at 1h. The primary endpoint of the study was survival to day 41 post-infection. A phase I study with five cohorts of healthy volunteers administered AVI-7288 up to 16 mg/kg IV once a day for 14 day and monitored for adverse events to assess safety. Results: Long-term survival to 41 days post-infection in 83% (5/6), 83% (5/6), 100% (6/6), and 83% (5/6) in groups 1, 2, 3, and 4, respectively, were observed compared to 0% (0/6) survival in the untreated control group. AVI-7288 was well tolerated in healthy volunteers following daily doses of up to 16 mg/kg/day for two weeks. Conclusions: The PMOplus™ oligomer (AVI-7288) targeting NP provides survival benefit when administered for up to 96 hours post infection from MARV lethal challenge in cynomolgus macaques. AVI-7288 is well tolerated in healthy volunteers at doses up to 16 mg/kg/day.

178 Non-Polio Enterovirus Acute Flaccid Paralysis: The Need for Antiviral Drug Development
Olufunmilayo G. Oyero
University of Ibadan, Ibadan, Nigeria

Non-polio enteroviruses (NPEVs) are common and distributed worldwide causing diseases ranging from asymptomatic infections to occasional outbreaks in which a larger-than-usual number of patients develop clinical diseases, sometimes with fatal consequences. Of great importance is the acute flaccid paralysis (AFP), incidence of which has continued to grow in polio free regions. Currently there are no specific antiviral agents available for the therapy of enterovirus infection hence an insight into the antigenic diversity of NPEVs from different regions would be necessary for the development of globally protective vaccines. This study was therefore designed to determine the spectrum of NPEV serotypes associated with AFP in Nigeria with the aim of depositing the sequences to the growing database in the GenBank: Two stool samples each was obtained from 966 cases of AFP from 26 states of Nigeria, over a period of 3 years. Virus isolation and serotyping were carried out according to the WHO protocols. Full characterization was achieved using molecular typing methods based on reverse transcription polymerase chain reaction (RT-PCR) and nucleotide sequencing of the partial viral protein 1 (VP1) gene. Sequences were compared with those of human enterovirus reference strains and other picornaviruses available in the GenBank. Fifty-six NPEV isolates recovered were identified into echoviruses (E3, E6, E11, E14, E20, E24, E29, and E30) and coxsackie B viruses (CVB3, CVB5). However the derived VP1 sequences of 32 Nigeria NP-AFP NPEVs have been fully characterized and deposited in the GenBank under the appropriate accession numbers (GQ496533 - GQ496548, GQ496551- GQ496556, GQ496567 – GQ496569, GQ496571- GQ496577). Phylogenetic tree constructed using the neighbor-joining method revealed genealogical relationships to viruses from other parts of the world. This study documenting the first report of NPEV sequences associated with acute flaccid paralysis in Nigeria is consistent with findings from other countries. The association of a wide spectrum of serotypes with NP-AFP poses a challenging problem toward development of effective anti-enteroviral vaccines. Hence the search for effective antiviral therapy is advocated.
179 Treatment of Influenza Patients with Aprotinin Aerosol Generated by Stationary and Metered Dose Manual Inhalers

Oleg P. Zhirnov¹, Natasha O. Bokova², Elena I. Isaeva¹, Irina V. Vorobjeva¹, Olga A. Saphonova², Nikolai A. Malyshev²

¹The D.I.Ivanovsky Institute of Virology, Moscow, Russia, ²1st Moscow Infectious clinics, Moscow, Russia

Aprotinin, a natural protease inhibitor from bovine lungs (TrasylolTM, ContrycalTM, etc.), is known to suppress a cleavage of influenza virus HA by host respiratory proteases and reduce virus replication in respiratory epithelium (for review see Zhirnov et al., Antiviral. Res. 92(1): 27-36 2011). Therapeutic and antiviral efficacies of inhalations of aprotinin aerosol generated either by stationary (SI) or meter dose manual (MDI) inhalers were studied in influenza patients. These clinical trials were performed during winter-spring outbreaks caused with seasonal H3N2 and pandemic Influenza H1N1pdm09 viruses. In studies with propellant A134-type MDI (AerusTM Russia), patients inhaled nasally 2 aerosol doses of aprotinin (160 Kallikrein-inhibiting Units (KIU)) each 2 hours for 5 days. In comparison group, patients were treated with IngavirinTM (a synthetic peptidoamine with unknown antiviral target), 90 mg per day for 5 days. On day 2 after treatment virus loads in nasal-pharyngeal washes were determined by real time PCR. About 10 fold decrease of virus load in aprotinin patients were determined in comparison to Ingavirin patients. Duration of clinical symptoms, such as rhinorrhea, weakness, headache, sore throat, cough, sore thorax, fever, was 1-2 days shorter in aprotinin then in ingavirin group. Similar therapeutic efficacy displaying a marked shortening of disease symptoms were achieved when influenza patients received 10 min inhalations 3 times daily for 5 days of aerosolized aprotinin solution generated in ultrasonic or ejector stationary nebulizers. Placebo patients received alkaline inhalations of 0,5% sodium bicarbonate generated in the same manner. About 300 patients were observed and no side effects and nasal-pharyngeal discomfort were documented in aprotinin-treated patients. Aerosolized aprotinin can be recommended as a drug of choice against Influenza caused by different viruses, including seasonal H1N1, H3N2, swine-like H1N1pdm09, and avian-like H7N9 viruses.

180 IND-directed Pharmacology and Toxicology of IQP-0528, a Novel HIV-1 Topical Microbicide

Christian Furlan-Freguia, Karen W. Buckheit, Anthony Ham, Robert W. Buckheit

ImQuest Biosciences, Frederick, USA

Worldwide nearly half of all individuals living with HIV are women who acquire the virus by heterosexual exposure. In the absence of a vaccine, topical microbicides are considered one of the best strategies for HIV prevention. Here, we describe the development of a novel microbicide, IQP-0528, formulated into a vaginal gel for the pre-exposure prophylaxis of sexually transmitted HIV. The efficacy and toxicity of IQP-0528 was evaluated using in vitro cell-based assays. IQP-0528 vaginal gel was characterized by rheological analysis. IND-enabling safety and toxicity studies were performed as per FDA recommendations. IQP-0528 is a small molecule pyrimidinedione with good chemical and metabolic stability and ease of synthesis. In vitro, IQP-0528 potently inhibits the replication of all clinical HIV subtypes (except O) with EC50 of 0.2-20nM and CC50 of 150-400uM. IQP-0528 maintains its activity in the presence of seminal and vaginal fluids with minimal toxicity to cell lines, explant tissues and vaginal flora. Formulation of IQP-0528 into a gel for vaginal delivery showed favorable physicochemical properties, long term stability, sub-nanomolar potency and minimal toxicity. IQP-0528 did not induce any mutations in the genotoxicity studies and did not cause acute toxicity in mice and rats. In rabbit and rat toxicity studies, IQP-0528 vaginal gel (up to 10% w/v concentration) dosed daily for 28 days was well tolerated with no adverse effects. In addition, little systemic absorption of IQP-0528 was observed when used at a clinical dose of 1%, while levels in vaginal tissue were in the ug range. IQP-0528 vaginal gel did not elicit skin sensitization reactions in guinea pigs and no teratogenic effects were observed in prenatal development studies. Finally, administration of IQP-0528 to dogs did not have any cardiovascular effects. The NOAEL of IQP-0528 is at least gel dose of 10%. In conclusion, we showed the significant safety and potency of IQP-0528 which represents a novel and ideal clinical microbicide candidate due to its high potency, ease of manufacturing, long-term stability, low toxicity, poor systemic but favorable local drug absorption and low cost of goods. Overall our data provide a rationale for continued advancement of this molecule to the clinic.
181 Epidemiological and Economic Modelling Study Demonstrating the Potential of Antiviral Agents to Control Classical Swine Fever Outbreaks

Robert Vrancken1, Jantien Backer2, Johan Neyts1,3, Nesya Goris1
1Okapi Sciences NV, Heverlee, Belgium, 2Central Veterinary Institute of Wageningen UR, Lelystad, Netherlands, 3Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Classical swine fever (CSF) is a highly contagious disease in pigs resulting in severe economic damages when introduced in a domestic pig population. Although currently many parts in the world are free of CSF, the disease is a continuous threat to pig populations free of disease without vaccination. In the event of a CSF incursion in the EU, minimal control measures are imposed, but often prove insufficient to mitigate the epidemic, especially in densely-populated pig areas. Alternative strategies such as pre-emptive culling encounter ethical objections, and emergency vaccination leads to prolonged export restrictions. Antiviral agents, however, are logistically easier to apply, provide instantaneous protection without inducing an antibody response, enabling standard diagnostic testing to detect (previously) infected animals. In this study, the effectiveness of an antiviral containment strategy was evaluated and compared to the more conventional approaches highlighted above, using a model describing within- and between-herd CSF virus transmission. Epidemics were simulated in one of the most densely-populated pig area in The Netherlands, with farms of varying sizes and pig types. The epidemiological modelling results showed that both an emergency vaccination policy and an antiviral containment strategy in a 2-km radius around detected premises are more effective than pre-emptive culling in a 1-km radius in reducing the size and the duration of the CSF outbreak. Moreover, the 2-km antiviral containment strategy proved as efficacious as the 2-km emergency vaccination policy. The epidemiological data were subsequently used to evaluate a number of CSF control strategies on their economic merits. Compared to the reference policies of pre-emptive culling and emergency vaccination, a control strategy based on the use of a potent, selective and safe antiviral agent is economically viable. In summary, both from an epidemiological and economic perspective, controlling CSF outbreaks using antiviral agents seems to be preferred.

EUVIRNA

185 Identification of Host Factors Involved in Lipid Droplet Homeostasis and the Replication of Hepatitis C and Dengue Virus by RNAi Screening

Ina Karen Stoeck1, Gualtiero Alvisi2, Sandeep Amberkar3, Narsis Kiani3, Christoph Sommer4, Wolfgang Fischl1, Marion Poenisch1, Fred A. Hamprecht4, Giorgio Palù2, Lars Kaderali3, Ralf Bartenschlager1
1Department of Infectious Diseases, Heidelberg University, Heidelberg, Germany, 2Department of Molecular Medicine, University of Padua, Padua, Italy, 3Institute of Medical Informatics & Biometry, Dresden University of Technology, Dresden, Germany, 4Heidelberg Collaboratory for Imaging Processing, Heidelberg University, Heidelberg, Germany

Lipid droplets (LDs) are traditionally viewed as neutral lipid storage organelles. However, recent discoveries in LD biology have revealed their importance in several aspects of metabolic diseases and have also implicated their important, yet poorly defined, function in the replication cycle of diverse human pathogens such as the members of the Flaviviridae, the Hepatitis C Virus (HCV) and the Dengue Virus (DENV). Hence, the potential of lipid metabolic enzymes as drug targets for treatment of viral infections has been underrated. Keeping this in mind, we attempted to identify cellular pathways regulating LD homeostasis as well as the replication cycle of two LD-dependent viruses such as HCV and DENV. We conducted a RNA interference-based screen using a custom-made library targeting 230 genes potentially involved in LD homeostasis. Alterations of LD properties were scored by quantifying LD distribution, size and lipid content whereas the impact on DENV and HCV was measured by using a whole virus-based method. We were able to confirm the involvement of 59 genes in LD homeostasis of which intriguingly 76% were additionally affecting at least one of the two viruses, supporting a pivotal role of LDs in their life cycle. Bioinformatic analyses revealed that cell cycle regulators, proteasome- and ubiquitin-dependent degradation pathways, as well as the COPI vesicle transport system act as central nodes controlling LD homeostasis and the replication cycle of either virus. A second deconvolution screen confirmed the involvement of the DEAD box helicase DDX3 and the calcium-independent phospholipase A2 beta (iPLA2beta) in the replication cycle of HCV. Follow-up studies proved an important role of DDX3 in HCV replication as well as of iPLA2beta in particle production.
187 Evaluation of Trans-complementation for Dengue Virus Serotype 2 Non Structural Protein 4B, a Target For Inhibition of Flavivirus Replication

Ilane Hernandez-Morales¹, Marnix Van Loock¹, Kai Dallmeier², Johan Neyts², Gregory Fanning¹

¹Janssen Infectious Diseases BVBA, Beerse, Belgium, ²Rega Institute, KU Leuven (#Equal contribution), Leuven, Belgium

Dengue is one of the most important mosquito-transmitted viruses worldwide. Epidemiological models estimate that it causes 390 million infections annually. DENV, a positive strand RNA virus from the flavivirus family, encodes seven non-structural (NS) proteins that are essential in viral replication. NS4B has been suggested to play an important role in viral replication by facilitating the formation of the replication complex, and in counteracting the innate immune response however, its exact function is still unclear. More recently, NS4B has been shown to be a promising target of several small molecule inhibitors of DENV replication as a novel means for antiviral intervention. We aimed at establishing a robust system to study the function of NS4B by means of restoring the replication of defective DENV genomes by trans-complementation. To that end a replication-defective DENV full infectious clone with a lethal P104R mutation in NS4B expressing green fluorescent protein (GFP) and a replication-competent sub-genomic replicon expressing the Renilla luciferase gene were co-transfected. Replication of both genomes was measured by either reporter activity. The negative control was a non-replicative DENV infectious clone expressing GFP, in which a stop codon replaced the start codon of the polymerase NS5. Our results show that NS4B P104R in the context of a full DENV genome cannot be trans-complemented neither in Huh7 nor BHK cells if transfected with a sub-genomic helper replicon at the same time. In conclusion, our data suggest that those functions of NS4B that are needed for viral replication have (preferably) to be provided in cis, and cannot readily be rescued in the presence of a second dengue replicon in the same cell. We will discuss our findings in the light of recently published evidence for the opposite (Zou, Xie et al. 2014), and what lack of trans-complementation might imply for the efficacy of NS4B targeting antivirals and development of drug resistance.

188 The Chikungunya Virus Replication Complex: In Vitro Characterization and Mode of Action Studies on Antiviral Compounds

Irina Albulescu, Ali Tas, Florine Scholte, Eric Snijder, Martijn van Hemert

Leiden University Medical Center, Leiden, Netherlands

Chikungunya virus (CHIKV) is a re-emerging mosquito-borne alphavirus that causes severe persistent arthralgia, for which registered vaccines or antiviral therapy are unavailable. Locally-transmitted infections in Italy, France and the 2013/14 outbreak in the Caribbean illustrate the increasing burden of CHIKV, also outside Africa and Asia, where it affected millions of people since 2006. CHIKV RNA synthesis relies on a replication and transcription complex (RTC) that – as for all +RNA viruses – is associated with intracellular membranes. To gain more insight into the molecular details of RNA synthesis, the mode of action of inhibitors, and their composition, we have isolated active CHIKV RTCs from infected cells. Their activity was characterized in vitro by measuring $^{32}$P-CTP incorporation into CHIKV RNA. We observed synthesis of genome, subgenomic RNA and a ‘jamming product’ (~7.5 kb 5’ fragment of genome) that was also found in infected cells. The activity was dependent on all 4 NTPs and an energy regenerating system and reaction products were mainly single-stranded RNA molecules. Active RTCs could be pelleted at 10,000 x g in a fraction that was enriched in nonstructural protein 1 (nsP1) and the RdRp nsP4, while the bulk of nsP2, nsP3 and capsid protein was found in other subcellular fractions (in line with their localization in immunofluorescence studies). Endogenous nuclease activity caused rapid degradation of control RNAs, but CHIKV RNAs and the active RTC appeared to be in a (membrane-) protected environment, a notion further supported by the results of protase and核酸ase protection experiments. A set of compounds that inhibit CHIKV replication in cell culture was tested in the in vitro RTC assay. In contrast to 3’dNTPs, which act as chain terminators and inhibited RTC activity, ribavirin-triphosphate and 6-azaUTP did not affect RTC activity in vitro, suggesting they are incorporated into viral RNA and may act in vivo as antimetabolite or by inducing lethal mutagenesis. In conclusion, our in vitro RTC assay is a useful tool for studying CHIKV RNA synthesis and the mode of action of antiviral compounds. Supported by EU-FP7 ITN EUVIRNA (grant 264286).
189 Investigation of Calicivirus Replication Complex Formation
Yuka Otsuka¹, Charlotte Melia², Montserrat Bárcena², Jacques Rohayem¹,³
¹Riboxx GmbH, D-01445, Radebeul, Germany, ²Leiden University Medical Center, Leiden, Netherlands, ³Institute of Virology, Medical Faculty, Technische Universität Dresden, Germany
Positive stranded RNA viruses are reported to induce membrane reorganization of host cells during infection. This reorganization is thought to provide an optimal environment for virus replication. Replication occurs at the replication complex (RC), which consists of viral and host proteins. In Calicivirus, formation of RC is not well understood. Viruses of the calicivirus family infect both humans and animals and are responsible for outbreaks of respiratory infections in cats (felines calicivirus, FCV) and food-borne gastroenteritis (human norovirus and sapovirus). Understanding the mechanisms underlying formation of the RC may lead to the development of new antiviral strategies against calicivirus. In this study, feline calicivirus (genus: Vesivirus, family: Caliciviridae) will be cultivated in Crandell-Rees Feline Kidney (CRFK) cells to investigate the viral replication complex. Using electron tomography we will establish the 3D morphology of FCV induced replication membranes.

190 Host Cell Cholesterol Landscape Is Important for Efficient Picornavirus Replication
Lucian Albulescu, Jeroen RPM Strating, Frank JM van Kuppeveld
Division of Virology, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht, Netherlands
Membrane rearrangement leading to replication organelles (ROs) formation is critical for the replication of picornaviruses. Lipids and lipid remodelling processes are considered of fundamental importance for RO formation. Strikingly, the involvement of lipids is largely obscure. Recently, phosphatidylinositol-4-phosphate (PI4P) was the first lipid discovered to be important for the replication of a number of picornaviruses, including Coxackievirus B3 (CVB3) and encephalomyocarditis virus (EMCV). Studies on viruses from different families (e.g. hepatitis C virus) have not only revealed a role for PI4P in virus replication and membrane remodelling, but also for the lipid cholesterol. Here, we set out to investigate the involvement of cholesterol in picornavirus replication. We found that cholesterol is redistributed to ROs in CVB3 and EMCV-infected cells and that cholesterol depletion impairs virus replication. Furthermore, we established that cholesterol shuttling rather than synthesis was required for efficient replication. Cholesterol shuttling was suggested to depend on oxysterol-binding protein-related proteins (ORPs) and their function is regulated by PI4P. Therefore, we tested ORP ligands and found that they have a strong inhibitory effect on picornavirus replication. Mutations that rendered viruses resistant to compounds that inhibit PI4P production also provided resistance against ORP ligands, further supporting the link between PI4P and ORPs. We conclude that picornaviruses require cholesterol for efficient replication and that ORP-mediated cholesterol shuttling might be important for picornavirus replication.

191 Development of Suitable Surrogate Models to Investigate the Mechanism of Action of Novel FMDV Inhibitors
Denny Kollanur¹, Lyre Espada Murao¹, Jan Swinnen¹, Annebel R De Vleeschauwer², David J Lefebvre², Kris De Clercq², Johan Neyts¹,³, Nesy Goris¹
¹Okapi Sciences NV, Heverlee, Belgium, ²Veterinary and Agrochemical Research Center (CODA-CERVA), Brussels, Belgium, ³Rega Institute for Medical Research, University of Leuven (KU Leuven), Leuven, Belgium
Foot-and-mouth disease (FMD) virus (FMDV), member of the genus Aphthovirus within the family Picornaviridae, is a highly contagious pathogen for cloven-hoofed livestock and wildlife animals. Its manipulation is restricted to high-containment BSL-3 laboratories, thus impeding the development of an antiviral containment strategy for FMD outbreaks. Two phylogenetically closely related viruses, equine rhinitis A virus (ERAV) and bovine rhinitis B virus (BRBV), were evaluated as potential surrogate models for FMDV antiviral studies. While there is to date no efficient cell culture system available for BRBV, we established a
viral RNA reduction assay using Madin-Darby bovine kidney cells that was used in subsequent antiviral tests. Antiviral tests based on the read-out of virus-induced cytopathic effects and viral RNA reduction assays were carried out on Vero A cells for the ERAV surrogate model. The surrogate models were evaluated using two broad-spectrum inhibitors of single-stranded positive-sense RNA viruses (2'-C-methylcytidine or 2CMC and ribavirin), a recently discovered potent non-nucleoside pan-serotypic FMDV-inhibitor (compound A), and an enterovirus-specific inhibitor (enviroxime). As expected, both 2CMC and ribavirin inhibited the surrogate aphthoviruses and enviroxime was devoid of activity against both ERAV and BRBV. Compound A exhibited antiviral activity against BRBV and ERAV. The surrogate models presented here are easily manipulated in BSL-2 laboratories and thus can be utilized for investigating the mechanisms of inhibition for novel anti-FMDV compounds.

192 Mapping of RNA Structural Elements in the Sapovirus Genome
Subash K. Rai1, Alexander P. Gultyaev2, Alexander E. Gorbalenya3,4, Jacques Rohayem1,5
1Riboxx GmbH, Pharmapark, Radebeul, Germany, 2Dept. of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands, 3Dept. of Med. Microbiology, Leiden University Medical Center, Leiden, Netherlands, 4Faculty of Bioengineering & Bioinformatics, Lomonosov Moscow State University, Moscow, Russia, 5Institute of Virology, Medical Faculty, Technische Universität Dresden, Dresden, Germany

Genome replication of viruses with a single-stranded positive-oriented RNA genome (+ssRNA) – such as poliovirus – relies on interaction of viral enzymes with genomic/antigenomic RNA structural elements. In this study, the presence of RNA structural elements in the +ssRNA genome of the sapovirus (Caliciviridae) was hypothesized. Sapovirus is responsible for outbreaks of gastroenteritis worldwide. Understanding the role of RNA structural elements implicated in sapovirus genomic replication could set the cornerstone for development of antiviral strategies to control sapovirus infections. To identify RNA structural elements of sapovirus, sequences of more than 30 full-length genomes of all known genotypes (n=6) were analyzed by applying multiple alignment and phylogenetic analysis (using VIRALIS platform) and RNA structure prediction algorithms (RNAz and RNAalifold). Overall, uneven and genotype-specific distribution of conserved RNA elements across the sapovirus genome were observed. At the 5’ terminal coding region (NS1-2) of sapovirus genomic RNA, a tetranucleotides loop (tetraloop) structural motif (cYCAGg) was identified. It is thermodynamically stable and evolutionary conserved in the majority of sapovirus strains. This tetraloop structural motif belongs to the cYNMGg motif family that also includes a tetraloop in the loop D of the 5’-end cloverleaf structure implicated in the control of replication in enteroviruses. Other RNA structural elements highly conserved in the sapovirus genome included tetraloop motif uUCYAa and Y-shaped structures at 3’ terminus. Also redundant RNA structural elements in different positions of antigenomic RNA were also observed and tentatively implicated in nucleotidylylation of VPg. This study is to our knowledge the first systematic mapping of RNA structural elements in the genome/antigenome of sapovirus, a major food-borne pathogen.

193 Shape-based Virtual Screening for the Identification Of Novel DENV Helicase Inhibitors.
Iuni M. L. Trist1, Suzanne Kaptein2, Pieter Leyssen2, Chanqing Li3, Johan Neyts2, Bruno Coutard3, Andrea Brancale1
1Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, 2Rega Institute of Medical Research, KU Leuven, Leuven, Belgium, 3AFMB, Aix Marseille Université, Marseille, France

The over 30-fold increase in incidence of the last 50 years has brought the number of registered cases of dengue virus (DENV) related illnesses to over 100 million every year. DENV is endemic in more than 100 countries and the spread of its mosquito vector in the USA and Europe is causing a big concern for human health worldwide. Three distinct DENV clinical pictures have been described: dengue fever, dengue haemorrhagic fever and dengue shock syndrome. The latter two have a more frequent fatal outcome and are commonly caused by a secondary infection with a different DENV serotype, of which 5 have been identified so far. Despite the huge effort of the last 10 years in antiviral research, there is still no specific anti-DENV drug available and, to date, vaccine development has proven to be very difficult. An essential
step in DENV replication is dsRNA unwinding, which is catalysed by the NTPase/helicase domain of the non-structural protein NS3. Based on ouabain structure, a natural glycoside that was found to inhibit DENV helicase activity, we have used a shape comparison approach to screen a virtual database of approximately 210,000 drug-like molecules. The compounds with the most similar shape to ouabain were purchased and evaluated for selective antiviral activity in a virus-cell-based and enzyme-based assays. The two most promising compounds that inhibited the helicase-RNA binding with low µM IC₅₀ were further developed. In this presentation, we will discuss the molecular modelling study and the biological results that have been obtained in relation to their implications in antiviral drug design.

194 Distinct Picornaviruses Rely on Distinct PI4Ks for Replication
Cristina Dorobantu, Hilde van der Schaar, Frank van Kuppeveld
Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Virology Division, Utrecht, Netherlands

All positive-strand RNA viruses generate specialized membranous structures with unique lipid and protein composition for the purpose of genome replication. The functionality of these so-called “replication organelles” depends on the concerted actions of both viral non-structural proteins and co-opted host factors. Several RNA viruses hijack PI4Ks (phosphatidylinositol-4-kinases) to generate their replication organelles. For example, the flavivirus hepatitis C virus depends mainly on the ER-localized PI4KIIα, while enteroviruses and kobuviruses (family Picornaviridae) hijack the host factor PI4KIIβ for replication. The small membrane-anchored enteroviral protein 3A is responsible for the recruitment of PI4KIIβ, yet the underlying mechanism has remained elusive. Recently, it was shown that kobuviruses recruit PI4KIIβ through the interaction with ACBD3 (acyl-CoA-binding protein domain 3), a novel interaction partner of PI4KIIβ. Enterovirus 3A was previously proposed to recruit PI4KIIβ via the upstream factors GBF1/Arf1. Here, we investigated whether enteroviruses recruit PI4KIIβ through ACBD3 or GBF1/Arf1. Our findings indicate that, unlike originally envisaged, the enterovirus coxsackievirus recruits PI4KIIβ to replication organelles independently of ACBD3 and GBF1/Arf1. Whether 3A recruits PI4KIIβ via a direct interaction or via another host factor remains an open question. Additionally, we show that mengovirus of the distantly related genus Cardiovirus within the Picornaviridae family does not rely on PI4KIIβ, but instead requires the ER-localized PI4KIIIα for replication. Depletion as well as chemical inhibition of PI4KIIβ drastically reduced mengovirus replication, while inhibition of PI4KIIβ had no effect. Currently, we are further investigating how PI4KIIIα and PI4P function in cardiovirus replication.

195 Venezuelan Equine Encephalitis Virus nsP1: From Functional Characterization to Understanding of Antiviral Mechanism of Action.
Changqing Li, Jaime Guillén, Julie Lichière, Bruno Canard, Etienne Decroly, Bruno Coutard
Centre National de la Recherche Scientifique, Aix-Marseille Université, CNRS UMR 7257, AFMB, Marseille, France

Venezuelan equine encephalitis virus (VEEV) is an infectious pathogen belonging to the New World alphaviruses. This arbovirus is responsible for encephalitis in animals and humans. Its genomic and sub-genomic RNAs are protected by a 5′ end cap structure, which is essential for translation of viral proteins. The synthesis of cap structures in alphavirus is a multi-step reaction mainly driven by nsP1 that methylate a GTP molecule at its N7 position. The N7-GTP then forms a covalent link with nsP1, releasing PPI. This N7 Gp-nsP1 is known as a guanylyltransfer intermediate. The functional characterization of VEEV nsP1 is an important step for the development of new antiviral strategies based on specific inhibition of this capping enzyme. To that aim, VEEV nsP1 was produced in E.coli and purified by two steps of affinity chromatography. Both methyltransferase (MTase) and Guanylyltransferase (GTase) activities were characterized and tools were developed in order to uncouple both reactions. The critical amino acids positions for both MTase and GTase functions were next identified by mutagenesis. Finally we developed an immunoassay to follow the inhibition properties of S-adenosyl methionine (SAM) analogues on nsP1. Altogether, this study allowed the individual characterization of the MTase and GTase activities carried by VEEV nsP1 and demonstrated for the first time that small molecules, such as sinefungin, can be used as starting point for antiviral strategies.
196 The Conserved N-terminal Domain Associated with the Nidovirus RNA Polymerase Contains Residues Essential for its Nucleotidylation

Kathleen C Lehmann1, George M C Janssen2, Peter A van Veelen2, Eric J Snijder1, Alexander E Gorbalenya1,3, Clara C Posthuma1

1Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands, 2Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands, 3Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia

RNA viruses employ multifunctional proteins as a result of considerable constraint on their genome size. We investigated a so far uncharacterized domain associated with the RNA polymerase of the (+)RNA viruses of the order Nidovirales (arteri-, corona-, mesoni- and roniviruses). Bioinformatics analyses showed that this N-terminal domain is conserved in all known nidoviruses, making it a second molecular marker of this order. In agreement with this observation, alanine substitution of conserved residues of the domain’s three sequence motifs in the nsp9 protein of our model arterivirus equine arteritis virus (EAV) produced either non-viable or crippled mutants, with reversion being observed for the latter group. Given the lack of appreciated sequence similarity of this domain with characterized protein families, we considered the chemical nature of its few conserved residues, which suggested an enzymatic function. We reasoned that an N-terminally encoded activity may be connected to known nidovirus enzymes involved in RNA synthesis or processing. Accordingly, we demonstrate for the first time nucleotidylation activity of recombinant EAV nsp9, which was severely diminished upon alanine substitution of conserved amino acids in the N-terminal domain. This activity was detected with GTP and UTP, with UTP being the preferred substrate. Although we could not localize the catalytic residue of this activity, mass spectrometry results indicated the presence of a nucleotide binding site within the N-terminal domain of nsp9. Together with the in vitro and reverse genetics mutagenesis data, this result supports an assignment of the nucleotidylation activity to the conserved N-terminal sequence motifs. In which of the three possible reactions, nucleic acid ligation, mRNA capping, or protein-primed RNA synthesis, this activity takes part and what its role in viral replication is remain the subjects of further studies.

197 Proofreading and Ribavirin-5’-monophosphate Excision by SARS-CoV Polymerase/exonuclease Complex

Lorenzo Subissi, Etienne Decroly, Isabelle Imbert, Bruno Canard
AFMB UMR7257 CNRS and Aix-Marseille University, Marseille, France

In the last decade, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) have emerged as human pathogens. These zoonotic coronaviruses cause respiratory diseases with a high case-fatality rate during epidemics in 2002-2003 and in 2013 to the present, respectively. These events emphasize the likelihood of a future emergence/re-emergence of a coronavirus. No treatment is yet available. SARS-CoV is resistant to the action of the broad-spectrum antiviral ribavirin (RBV). This nucleoside analogue is well known for its pleiotropic antiviral action. In the cell, RBV is phosphorylated upon entry and interferes with innate immunity regulation. Ribavirin 5’-monophosphate (RMP) inhibits inosine monophosphate dehydrogenase (IMPDH) by reducing the intracellular guanosine triphosphate (GTP) pool. In parallel, ribavirin 5’-triphosphate (RTP) can be incorporated into viral genomic RNA, potentially triggering mutational events.

Coronaviruses have RNA genomes of outstanding length (up to 32 kb). Their replication/transcription is mediated by the canonical RdRp (nsp12), which binds to a processivity factor, made of the nsp7/nsp8 complex, to synthesize RNA. This polymerase complex (hereafter SARS-Pol) can also bind to nsp14, a bi-functional enzyme with 3’->5’ exonuclease (ExoN) and N7-cap methyltransferase activities.

Through direct sequencing, we provide evidence that SARS-CoV nsp14-ExoN is recruited by SARS-Pol to coordinate a DNA-polymerase-like proofreading mechanism previously undescribed in the RNA virus world. Using this in vitro system we characterized the fidelity of SARS-Pol complex. Our results indicate that polymerase-induced nucleotide misincorporation reduces the polymerization rate up to 30-fold in vitro. This polymerisation rate decay opens a kinetic window allowing mismatch excision by the nsp14-ExoN activity. SARS-Pol can then resume polymerisation under a processive manner. We also demonstrate that RMP is preferentially incorporated into RNA opposite pyrimidines. Nsp14-ExoN is then able to excise the incorporated RMP, possibly accounting for the lack of antiviral activity of RBV against SARS-CoV.
**198** 

**Host-Pathogen Interaction as a Potential Target for Development of Antivirals: Role of SUMOylation in DENV Life Cycle.**

Joanna J. Zmurko, Johan Neyts, Kai H. Dallmeier  
*KU Leuven, Leuven, Belgium*


**199** 

**A Coxsackievirus Mutant Facilitates an Alternative Site for Replication Under PI4KIIIβ Inhibition**

Charlotte Melia¹, Hilde M. van der Schaar², Ronald W. A. L. Limpens¹, Eric J. Snijder¹, Abraham J. Koster¹, Frank J. M. van Kuppeveld², Montserrat Bárcena¹  
¹Leiden University Medical Center, Leiden, Netherlands, ²University of Utrecht, Utrecht, Netherlands

Positive-sense RNA viruses reorganize intracellular membranes into novel membranous structures suitable for replication of their genome. These replication structures are formed by both viral proteins and hijacked host cell factors. Enteroviruses, belonging to the family of *Picornaviridae*, rely on the host cell factor PI4KIIIβ (phosphatidylinositol-4-kinase type IIIβ), since depletion by siRNA or pharmacological inhibition of this kinase strongly reduces replication. Surprisingly, the enterovirus coxsackievirus B3 (CVB3) can overcome PI4KIIIβ depletion or inhibition by the introduction of a single point mutation (H57Y) in the viral protein 3A. How the 3A-H57Y mutant is able to bypass this need for PI4KIIIβ remains unknown. Using electron microscopy (EM) we explored the effect of PI4KIIIβ inhibitors on cell ultrastructure in CVB3 wild type (WT) or CVB3 3A-H57Y (mutant) infection. For optimal sample preservation, high-pressure freezing/freeze substitution was carried out for all EM experiments. We found that cells infected with mutant CVB3 follow a typical pattern of replication structure development. For optimal sample preservation, high-pressure freezing/freeze substitution was carried out for all EM experiments. We found that cells infected with mutant CVB3 follow a typical pattern of replication structure development. Early structures are tubular, interspersed with closed double-membrane vesicles (DMVs). As infection progresses tubules disappear and are replaced with large fields of DMVs that extend throughout the cytoplasm. This progression is identical to that of WT CVB3 infection. Golgi and replication structures were never seen simultaneously in cell sections analysed, consistent with suggestions that replication structures are derived from the secretory pathway. In the presence of PI4KIIIβ inhibitors WT replication does not occur and, accordingly, typical replication structures were absent. Remarkably, while the 3A-H57Y mutant was able to replicate under PI4KIIIβ inhibition, neither tubules nor regions of DMVs were detected. These data suggest that, under PI4KIIIβ-inhibitory conditions, the 3A-H57Y mutant adopts a strategy that entirely bypasses the need for typical replication structures. This has important implications for our understanding of replication in all positive-sense RNA viruses.
200 Comparative Study of the Anti-enterovirus 71 Activity of a Selected Series of Enterovirus Inhibitors

Aloys Tijmsa1, David Franco1, Rolf Hilgenfeld2, Mathy Froeyen1, Johan Neyts1
1Rega Institute for Medical Research, Leuven, Belgium, 2University of Lübeck, Lübeck, Germany

Enterovirus 71 (EV71) frequently causes outbreaks of Hand, Foot and Mouth Disease, sometimes leading to life-threatening complications such as aseptic meningitis, encephalitis, pulmonary edema and viral myocarditis. Currently, treatment of EV71 infected patients is symptomatic as no specific antiviral treatment exists. We here report on a comparative study of the in vitro antiviral activity of (i) the enterovirus protease inhibitors (PI) rupintrivir and SG85 (ii) the replication inhibitor enviroxime and (iii) the capsid-binders pirodavir and pleconaril against a representative panel of 21 EV71 isolates. Rupintrivir, SG85 and enviroxime potently inhibited all isolates. Strains of subgenogroup C2 and C4 were more sensitive to the PI’s than those belonging to subgenogroup B5. Interestingly, whereas pirodavir proved highly active against all strains, pleconaril was completely devoid of any activity. An in silico docking study revealed that pleconaril was, unlike pirodavir, unable to anchor in the canyon under the receptor binding pocket. Our data may help to design potent and pan-EV71 antivirals and will serve as a reference panel against which novel inhibitors can be profiled.

201 Characterization of the Mode-of-action of a Potent Dengue Virus Capsid Inhibitor

Pietro Scaturro1, Iuni Trist2, David Paul1, Chelsea M. Byrd3, Robert Jordan3, Andrea Brancale2, Ralf Bartenschlager1,4
1Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany, 2School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, 3SIGA Technologies, Inc., Corvallis, USA, 4German Center for Infection Research, Heidelberg, Germany

Infections with the Dengue virus (DENV) represent a significant burden for global health, with more than 100 million cases reported every year worldwide, and increasing number of patients developing life-threatening disease. Despite the interest in developing vaccine candidates and anti-viral compounds, there is currently a lack of approved therapeutics for the treatment of DENV infections. We have previously reported the identification of ST-148, a small molecule inhibitor that exhibited broad and potent anti-viral activity against all DENV serotypes and significantly reduced viremia in vivo. In the present study, we investigated the mode-of-action of this promising compound by using a combined approach involving biochemical and imaging-based techniques. We confirmed that ST-148 targets the Capsid protein and obtained evidence of a bi-modal anti-viral activity affecting both entry and assembly/release of DENV viral particles. We further demonstrated that ST-148 does not influence the co-localization of Capsid with lipid droplets or dsRNA, but induced Capsid accumulation in nuclear, insoluble aggregates. Additionally, by using a robust BRET-based assay we observed a dose-dependent increase of Capsid self-interaction upon treatment with ST-148, and further corroborated these results by molecular modeling studies. Taken together, our findings suggest that ST-148 exerts its anti-viral activity by preventing disassembly of incoming and assembly of new virus particles, thus unraveling a novel promising target for small molecule inhibitors of DENV.
202 Crimean-Congo Hemorrhagic Fever Virus and Ebola Virus Infection of Humanized Mice Leads to Prominent Antiviral Mediator Induction

Ayan K. Chakrabarti1, Brian H. Bird1, Clifton P. Drew2, Ute Stroehler1, Stuart T. Nichol1, Christina F. Spiropoulou1

1Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, USA, 2Infectious Disease pathology Branch, Centers for Disease Control and Prevention, Atlanta, USA

Viral hemorrhagic fevers (VHFs) are a diverse set of high consequence pathogens causing a spectrum of highly lethal diseases including Ebola (EBOV Family Filoviridae, Genus Ebolavirus) and Crimean-Congo hemorrhagic fever (CCHFV Family Bunyaviridae, Genus Nairovirus). Infection with either virus leads to high case morbidity/mortality, yet each has a distinct ecological niche, transmission modality, and molecular pathogenesis. Laboratory based studies of the underlying virulence mechanisms are problematic. EBOV isolates derived directly from human specimens do not cause disease in immunocompetent adult mice without serial passage and adaptation. More strikingly, despite decades of effort with multiple species of immunocompetent laboratory animals including non-human primates, CCHFV disease occurs only in humans. To overcome these limitations, we sought to determine if BLT mice (NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ) with engrafted human immune systems could be infected with isolates of Ebola virus (Zaire Mayinga 1976) or strains of CCHFV (Oman1998, Turkey2004) derived directly from fatal human clinical specimens. BLT mice succumbed to lethal infection with high virus loads and histopathology that was consistent with data from lethal human cases. EBOV infected mice responded with broad-spectrum induction of pro-inflammatory antiviral mediators (i.e., IL-6, IL-8, TNFa, ISG15, CXCL10) consistent with human patients. In contrast, expression of pro-inflammatory mediators among CCHFV infected mice was less pronounced even though both viruses led to uniformly lethal outcomes. This study represents the first time that lethal disease has been observed in any immune competent animal infected with CCHFV and provides an opportunity for further investigation of CCHFV and non-rodent adapted EBOV virulence, immunopathogenesis, and assessment of anti-viral therapeutics in the context of a humanized immune system.

LATE BREAKER

LB1 Potent Broad Spectrum Anti-DNA Viral Activity and PK Data for HPMPA and HPMPC Tyrosinamide Prodrugs

Charles E. McKenna1, Boris A. Kashemirov1, Ivan Krylov1, Melissa M. Williams1, Mark N. Prichard2, Caroll Hartline2, Emma Harden2, Geraldine Jefferson2, Kathy Keith2, John M. Hilfinger3, Elke Lipka3, Mindy Collins3, Dawn Reyna3

1Department of Chemistry, University of Southern California, Los Angeles, CA, USA; 2Department of Pediatrics, University of Alabama Birmingham, Birmingham, AL, USA; 3TSRL, Inc., Ann Arbor, MI, USA

V9-[(2S)-3-Hydroxy-2-phosphonomethoxy-propyl]adenine ((S)-HPMPA) and cidofovir ((S)-HPMPC, Vistide®), are acyclic nucleoside phosphonates (ANPs) that exhibit antiviral activity against many DNA viruses. An inherent drawback of these drugs is the presence of an ionizable phosphonic acid group, which results in poor cell membrane permeability and low oral bioavailability at physiological pH. We previously reported the synthesis and structure-activity relationship studies of a series of lipophilic tyrosinamide prodrugs of (S)-HPMPA and (S)-HPMPC. These compounds displayed significantly enhanced antiviral activity against HCMV, cowpox and vaccinia virus in plaque reduction assays and an order of magnitude greater increased oral bioavailability compared to the parent drugs in a murine model. We present here in vitro assay results for this series of tyrosinamide prodrugs of HPMPA and HPMPC against HSV-2, VZV and adenovirus together with pharmacokinetic distribution data in a murine model.

ACKNOWLEDGEMENTS: This work was supported by NIH Grant R43 AI100401 and DMID-NIAID-NIH-DHHS contract HHSN272201100016l.
**LB2** Synthesis And Antiviral Activities Of 3-Deaza-3-Fluoroaristeromycin And Its 5’-Homo and 5’-Nor Analogues  
Qi Chen, Chong Liu, and Stewart W. Schneller  
Molette Laboratory for Drug Discovery, Department of Chemistry and Biochemistry, Auburn University, Auburn, Alabama, USA  
The naturally occurring adenine based carbocyclic nucleosides aristeromycin and neplanocin A and their 3-deaza analogues have found a prominent place in the search for diverse antiviral agent scaffolds because of their ability to inhibit S-adenosylhomocysteine (AdoHcy) hydrolase. Following the lead of these compounds, we have prepared and had assayed their 3-fluoro-3-deaza analogues. The novel syntheses of these compounds and their significant antiviral properties (for example, towards monkeypox and measles both with IC\textsubscript{50} <0.32 μM and SI >313) will be presented.  
ACKNOWLEDGMENTS. We are grateful to the Molette Fund and Auburn University for support and to Gloria Kozamin of Microbiotix, Inc. Worcester and the NIAID in vitro antiviral assay team for the data presented.

**LB3** Ribavirin Treatment Failure in Chronic Hepatitis E is Associated with a Polymerase Variant with Increased Fitness  
Johan Neyts\textsuperscript{1}, Yannick Debing\textsuperscript{1}, Anett Gisa\textsuperscript{2}, Kai Dallmeier\textsuperscript{1}, Sven Pischke\textsuperscript{3}, Birgit Bremer\textsuperscript{2}, Michael Manns\textsuperscript{2}, Heiner Wedemeyer\textsuperscript{2}, Pothakamuri Venkata Suneetha\textsuperscript{2}  
\textsuperscript{1}Rega Institute for Medical Research, Department of Microbiology and Immunology, University of Leuven, Belgium; \textsuperscript{2}Department of Gastroenterology, Hepatology and Endocrinology, Center of Internal Medicine, Hannover  
ViChronic hepatitis E is often treated with extended courses of ribavirin and treatment failure has been occasionally observed. Here we report on two transplant patients chronically infected with the hepatitis E virus (HEV) who underwent extensive ribavirin therapy, but failed to clear the virus. In one case, the viral load decreased initially but viral rebound was noted 4 months after start of treatment and consequently the patient developed fatal decompensated liver disease. The second patient experienced twice a relapse following ribavirin treatment (which was for 5 and 8 months respectively), despite undetectable virus at the end of each treatment period. The viral genome was sequenced at various time points. For both patients, a single G-to-A nucleotide substitution resulting in a G1634R mutation in the viral polymerase was associated with ribavirin failure. This mutation was engineered into a genotype 3 (gt3) subgenomic HEV replicon. The mutant replicon proved equally sensitive to the antiviral activity of ribavirin as the wild-type construct. However, the 1634R replicon (and also a 1634K mutant) replicated markedly more efficiently than the wild-type, suggesting an increased replication fitness. This was confirmed by analysis of the growth kinetics of full-length (gt3) virus. Similar results were obtained with a gt1 replicon. In direct competition assay, the full length gt3 1634R mutant was able to out compete the wild type virus. In conclusion, we report on a HEV polymerase mutation that is associated with ribavirin treatment failure and that results in an increased in vitro replication fitness.
### Author Index

| A | Abell, L | 91 |
| Agnihotram, S | 17, 176 |
| Ahmed, H | 130 |
| Alayli, F | 100 |
| Albulescu, I | 95, 209 |
| Albulescu, L | 211 |
| Alekseeva, E | 62 |
| Alexandre, A | 35 |
| Ali, M | 25 |
| Alvisi, G | 206 |
| Amano, M | 83 |
| Amberkar, S | 206 |
| Andrabi, R | 130 |
| Andrei, G | 24, 57, 94 |
| Andrews, Ph.D., W | 3 |
| Andrews, M | 26, 76 |
| Aoki, M | 83 |
| Appelbaum, J | 101 |
| Arico-Muendel, C | 142 |
| Arrildt, K | 102 |
| Artemenko, A | 62 |
| Aschenbrenner, L | 161 |
| Barnard, D | 93, 103, 105 |
| Bartenschlager, R | 222 |
| Barzinsky, V | 33 |
| Basok, S | 62 |
| Bassetto, M | 82 |
| Basu, A | 93 |
| Bavari, S | 53, 177 |
| Bedard, K | 104 |
| Bednar, M | 28 |
| Beigelman, L | 55, 125 |
| Bell, T | 29, 37 |
| Benschop, K | 30 |
| Bergstrom, T | 90 |
| Bergström, T | 146 |
| Bernstein, D | 106 |
| Biliavska, L | 31 |
| Bird, B | 202 |
| Bitko, V | 106 |
| Blasco, R | 24 |
| Bledsoe, R | 142 |
| Block, T | 40, 47, 119 |
| Boczar, A | 32, 35, 48 |
| Bogomolny, B | 33 |
| Bokova, N | 179 |
| Bonfanti, J | 44 |
| Bose, S | 21 |
| Bowlin, T | 93 |
| Boycott, H | 77 |
| Brancale, A | 42, 82, 214, 222 |
| Bravo, F | 106 |
| Bream, J | 129 |
| Brockman, M | 160 |
| Broker, T | 16 |
| Brown, K | 52, 118 |
| Brumme, Z | 160 |
| Buchholz, C | 32, 34, 35, 39, 145 |
| Buckheit Jr., R | 32, 34, 35, 64, 145 |
| Buckheit, Jr, R | 50 |
| Buckheit, K | 32, 34, 35, 48, 180 |
| Buckheit, R | 48, 49, 180 |
| Budgeon, L | 112 |
| Bunnell, B | 116 |
| Burger-Calderon, R | 54 |
| Busath, D | 77 |
| Byrd, C | 222 |
| Bárcena, M | 220 |
| C | Cai, C | 75 |
| Canard, B | 216, 218 |
| Cancellieri, Ph.D., M | 205 |
| Cancellieri, M | 82 |
| Cao, K | 108 |
| Cardin, R | 106 |
| Carlson, R | 157 |
| Chakrabarti, A | 202 |
| Challa, S | 107, 161 |
| Chamoun-Emanuelli, A | 36, 86 |
| Chandramohan, M | 99 |
| Chang, Ph.D., K | 9 |
| Chang, J | 40, 47, 119 |
| Charleston, J | 177 |
| Chattopadhyay, D | 27 |
| Chawla, R | 37 |
| Chen, G | 45 |
| Chen, H | 108 |
| Chen, L | 124 |
| Chen, T | 108 |
| Chen, W | 109 |
| Chen, Z | 36, 86 |
| Cheng, X | 75 |
| Cheshenko, N | 122 |
| Chhabra, M | 110 |
| Chiang, C | 95 |
| Chiragadze, Ph.D., N | 4 |
| Choi, J | 56 |
| Chou, D | 77 |
| Chow, L | 16 |
| Christensen, N | 111, 112 |
| Chu, C | 72, 115 |
| Chu, H | 56 |
| Chupikova, A | 57 |
| Cihlar, T | 22 |
| Cladel, N | 111, 112 |
| Clotet, B | 143 |
### Author Index

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clyde, J</td>
<td>137</td>
</tr>
<tr>
<td>Cohen, M.D., M</td>
<td>6</td>
</tr>
<tr>
<td>Cohen, M</td>
<td>28</td>
</tr>
<tr>
<td>Colacino, J</td>
<td>45</td>
</tr>
<tr>
<td>Coleman, C</td>
<td>41</td>
</tr>
<tr>
<td>Colpitts, C</td>
<td>113</td>
</tr>
<tr>
<td>Coluccia, A</td>
<td>42</td>
</tr>
<tr>
<td>Corona, A</td>
<td>114</td>
</tr>
<tr>
<td>Cosconati, S</td>
<td>114</td>
</tr>
<tr>
<td>Cotelle, P</td>
<td>75</td>
</tr>
<tr>
<td>Coutard, B</td>
<td>164, 214, 216</td>
</tr>
<tr>
<td>Creech, K</td>
<td>52</td>
</tr>
<tr>
<td>Cuconati, Ph.D., A</td>
<td>10</td>
</tr>
<tr>
<td>Cuconati, A</td>
<td>47</td>
</tr>
<tr>
<td>Cunningham, A</td>
<td>156</td>
</tr>
<tr>
<td>Cunningham, M</td>
<td>21</td>
</tr>
<tr>
<td>Donaldson, E</td>
<td>176</td>
</tr>
<tr>
<td>Dorobantu, C</td>
<td>215</td>
</tr>
<tr>
<td>Downs, B</td>
<td>174</td>
</tr>
<tr>
<td>Drach, Ph.D., J</td>
<td>15</td>
</tr>
<tr>
<td>Drach, J</td>
<td>117</td>
</tr>
<tr>
<td>Drew, C</td>
<td>202</td>
</tr>
<tr>
<td>Du, Y</td>
<td>40, 47</td>
</tr>
<tr>
<td>Duizer, E</td>
<td>30</td>
</tr>
<tr>
<td>Duprex, W</td>
<td>136</td>
</tr>
<tr>
<td>Duraffour, S</td>
<td>24</td>
</tr>
<tr>
<td>Dyall, J</td>
<td>41</td>
</tr>
<tr>
<td>Dyatkina, N</td>
<td>55, 125</td>
</tr>
<tr>
<td>Ekblad, M</td>
<td>146</td>
</tr>
<tr>
<td>Ellis, J</td>
<td>157</td>
</tr>
<tr>
<td>Engel, D</td>
<td>65</td>
</tr>
<tr>
<td>Esposito, F</td>
<td>114</td>
</tr>
<tr>
<td>Este, J</td>
<td>143</td>
</tr>
<tr>
<td>Esté, J</td>
<td>42</td>
</tr>
<tr>
<td>Fedchuk, A</td>
<td>59</td>
</tr>
<tr>
<td>Famigliini, V</td>
<td>42</td>
</tr>
<tr>
<td>Fan, J</td>
<td>161</td>
</tr>
<tr>
<td>Fanning, G</td>
<td>218</td>
</tr>
<tr>
<td>Fattom, A</td>
<td>106</td>
</tr>
<tr>
<td>Faulds, D</td>
<td>103, 105</td>
</tr>
<tr>
<td>Favorov, O</td>
<td>46</td>
</tr>
<tr>
<td>Favre, D</td>
<td>52</td>
</tr>
<tr>
<td>Fedchuk, A</td>
<td>33</td>
</tr>
<tr>
<td>Fedida, D</td>
<td>77, 160</td>
</tr>
<tr>
<td>Fedchuk, A</td>
<td>62</td>
</tr>
<tr>
<td>Feng, J</td>
<td>79</td>
</tr>
<tr>
<td>Ferris, M</td>
<td>134</td>
</tr>
<tr>
<td>Ferris, R</td>
<td>52, 118</td>
</tr>
<tr>
<td>Fischl, W</td>
<td>206</td>
</tr>
<tr>
<td>Fletcher, Pharm.D., C</td>
<td>171</td>
</tr>
<tr>
<td>Foster, G</td>
<td>21</td>
</tr>
<tr>
<td>Franco, D</td>
<td>78, 221</td>
</tr>
<tr>
<td>Frieman, M</td>
<td>41</td>
</tr>
<tr>
<td>Froeyen, M</td>
<td>221</td>
</tr>
<tr>
<td>Fung, A</td>
<td>125</td>
</tr>
<tr>
<td>Furlan-Freguia, C</td>
<td>180</td>
</tr>
<tr>
<td>Furuta, Y</td>
<td>18</td>
</tr>
</tbody>
</table>

**G**

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Page Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grydina, T</td>
<td>59</td>
</tr>
<tr>
<td>Gabuzda, D</td>
<td>87</td>
</tr>
<tr>
<td>Gaetzel, M</td>
<td>129</td>
</tr>
<tr>
<td>Galabov, A</td>
<td>155</td>
</tr>
<tr>
<td>Galardi, C</td>
<td>52</td>
</tr>
<tr>
<td>Gale, Jr, M</td>
<td>104</td>
</tr>
<tr>
<td>Galvez, M</td>
<td>45</td>
</tr>
<tr>
<td>Gao, M</td>
<td>91</td>
</tr>
<tr>
<td>Garcia-Lerma, Ph.D., G</td>
<td>170</td>
</tr>
<tr>
<td>Gardner, P</td>
<td>110</td>
</tr>
<tr>
<td>Gartland, M</td>
<td>165</td>
</tr>
<tr>
<td>Geer, L</td>
<td>158</td>
</tr>
<tr>
<td>Gentry, B</td>
<td>117</td>
</tr>
<tr>
<td>Gerondelis, P</td>
<td>118</td>
</tr>
<tr>
<td>Ghosh, A</td>
<td>83</td>
</tr>
<tr>
<td>Giancotti, G</td>
<td>82</td>
</tr>
<tr>
<td>Glen, R</td>
<td>43</td>
</tr>
<tr>
<td>Go, Y</td>
<td>127</td>
</tr>
<tr>
<td>Gobel, J</td>
<td>85</td>
</tr>
<tr>
<td>Goetz, A</td>
<td>52</td>
</tr>
<tr>
<td>Goetz, M</td>
<td>47</td>
</tr>
<tr>
<td>Gollnest, T</td>
<td>84</td>
</tr>
<tr>
<td>Gomes, V</td>
<td>156</td>
</tr>
<tr>
<td>Gomolayoko, I</td>
<td>73</td>
</tr>
<tr>
<td>Gong, E</td>
<td>44</td>
</tr>
<tr>
<td>Gorbalenya, A</td>
<td>213, 217</td>
</tr>
<tr>
<td>Goris, N</td>
<td>181, 212</td>
</tr>
<tr>
<td>Gosh, A</td>
<td>176</td>
</tr>
<tr>
<td>Govindarajan, R</td>
<td>72</td>
</tr>
<tr>
<td>Gowen, B</td>
<td>18</td>
</tr>
<tr>
<td>Graci, J</td>
<td>45</td>
</tr>
<tr>
<td>Graham, R</td>
<td>17</td>
</tr>
<tr>
<td>Grawoig, D</td>
<td>46</td>
</tr>
<tr>
<td>Greenhough, R</td>
<td>74</td>
</tr>
<tr>
<td>Grydina, T</td>
<td>33</td>
</tr>
<tr>
<td>Gu, Z</td>
<td>45</td>
</tr>
<tr>
<td>Gudz, G</td>
<td>31</td>
</tr>
<tr>
<td>Guenther, R</td>
<td>50</td>
</tr>
<tr>
<td>Guilford, W</td>
<td>103, 105</td>
</tr>
<tr>
<td>Guillen, J</td>
<td>216</td>
</tr>
<tr>
<td>Guluyaev, A</td>
<td>213</td>
</tr>
<tr>
<td>Guo, F</td>
<td>40, 47, 119</td>
</tr>
<tr>
<td>Guo, J</td>
<td>40, 47, 119</td>
</tr>
<tr>
<td>Gómez, M</td>
<td>83</td>
</tr>
<tr>
<td>Görander, S</td>
<td>146</td>
</tr>
<tr>
<td>Author Index</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td><strong>H</strong></td>
<td></td>
</tr>
<tr>
<td>Haasbacher, E .................................................. 120</td>
<td></td>
</tr>
<tr>
<td>Hagen, F .......................................................... 115</td>
<td></td>
</tr>
<tr>
<td>Hahn, D ............................................................. 121</td>
<td></td>
</tr>
<tr>
<td>Ham, A .............................................................. 41, 53, 180</td>
<td></td>
</tr>
<tr>
<td>Hamasaki, T ......................................................... 163</td>
<td></td>
</tr>
<tr>
<td>Hamouda, T ......................................................... 106</td>
<td></td>
</tr>
<tr>
<td>Han, S ................................................................. 56</td>
<td></td>
</tr>
<tr>
<td>Haque, M ............................................................. 139</td>
<td></td>
</tr>
<tr>
<td>Harris, E .............................................................. 45</td>
<td></td>
</tr>
<tr>
<td>Hart, B ................................................................. 41</td>
<td></td>
</tr>
<tr>
<td>Harter, M ............................................................. 172</td>
<td></td>
</tr>
<tr>
<td>Hartman, T ............................................................ 49, 50</td>
<td></td>
</tr>
<tr>
<td>Hartmayer, C .......................................................... 120</td>
<td></td>
</tr>
<tr>
<td>Hayashi, H ............................................................ 83</td>
<td></td>
</tr>
<tr>
<td>Haystead, T ............................................................ 51</td>
<td></td>
</tr>
<tr>
<td>Heald, A ............................................................... 53, 177</td>
<td></td>
</tr>
<tr>
<td>Heise, M ............................................................... 134, 176</td>
<td></td>
</tr>
<tr>
<td>Helfrick, A ........................................................... 49</td>
<td></td>
</tr>
<tr>
<td>Hensley, L .............................................................. 41</td>
<td></td>
</tr>
<tr>
<td>Herdewijn, Ph.D., P .................................................. 81</td>
<td></td>
</tr>
<tr>
<td>Hernandez-Morales, I ................................................. 218</td>
<td></td>
</tr>
<tr>
<td>Herold, B ............................................................... 34, 122, 158</td>
<td></td>
</tr>
<tr>
<td>Hidaka, A ............................................................... 141</td>
<td></td>
</tr>
<tr>
<td>Hightower, K .......................................................... 118</td>
<td></td>
</tr>
<tr>
<td>Hildenbrand, S ........................................................ 120</td>
<td></td>
</tr>
<tr>
<td>Hilgenfeld, R ........................................................... 221</td>
<td></td>
</tr>
<tr>
<td>Hiscott, J ............................................................... 95</td>
<td></td>
</tr>
<tr>
<td>Holl, E ................................................................. 153</td>
<td></td>
</tr>
<tr>
<td>Hoogenwerf, M ......................................................... 30</td>
<td></td>
</tr>
<tr>
<td>Howe, M ................................................................. 51</td>
<td></td>
</tr>
<tr>
<td>Hruby, D ................................................................. 24</td>
<td></td>
</tr>
<tr>
<td>Hu, G ................................................................. 149</td>
<td></td>
</tr>
<tr>
<td>Hu, J ................................................................. 108, 111</td>
<td></td>
</tr>
<tr>
<td>Hu, M ................................................................. 166</td>
<td></td>
</tr>
<tr>
<td>Huang, J ............................................................... 149</td>
<td></td>
</tr>
<tr>
<td>Hurst, B ................................................................. 174</td>
<td></td>
</tr>
<tr>
<td><strong>I</strong></td>
<td></td>
</tr>
<tr>
<td>Iadonato, S ............................................................. 104</td>
<td></td>
</tr>
<tr>
<td>Idrees, M ............................................................... 25</td>
<td></td>
</tr>
<tr>
<td>Ilina, T ................................................................. 128</td>
<td></td>
</tr>
<tr>
<td>Imbert, I ............................................................... 218</td>
<td></td>
</tr>
<tr>
<td>Irlbeck, D .............................................................. 52</td>
<td></td>
</tr>
<tr>
<td>Irvin, S ................................................................. 122</td>
<td></td>
</tr>
<tr>
<td>Isaeva, E ............................................................... 179</td>
<td></td>
</tr>
<tr>
<td>Islam, S ............................................................... 138</td>
<td></td>
</tr>
<tr>
<td>Ito, W ................................................................. 70</td>
<td></td>
</tr>
<tr>
<td>Iversen, P ............................................................. 53, 177</td>
<td></td>
</tr>
<tr>
<td>Iyer, R ................................................................. 21, 123, 172</td>
<td></td>
</tr>
<tr>
<td>Izumi, T ............................................................... 140</td>
<td></td>
</tr>
<tr>
<td><strong>J</strong></td>
<td></td>
</tr>
<tr>
<td>Jahrling, P ............................................................ 41</td>
<td></td>
</tr>
<tr>
<td>Jang, Y ................................................................. 127</td>
<td></td>
</tr>
<tr>
<td>Jansen, G .............................................................. 217</td>
<td></td>
</tr>
<tr>
<td>Jefferies-Francis, L .................................................. 54</td>
<td></td>
</tr>
<tr>
<td>Jeffery, J ............................................................... 110</td>
<td></td>
</tr>
<tr>
<td>Jennische, E ........................................................... 146</td>
<td></td>
</tr>
<tr>
<td>Ji, J ................................................................. 131</td>
<td></td>
</tr>
<tr>
<td>Jiang, D ................................................................. 124</td>
<td></td>
</tr>
<tr>
<td>Jiao, Y ................................................................. 17</td>
<td></td>
</tr>
<tr>
<td>Jin, Z ................................................................. 55, 125</td>
<td></td>
</tr>
<tr>
<td>Jochmans, D ............................................................ 38, 173</td>
<td></td>
</tr>
<tr>
<td>Johansen, L ............................................................ 41</td>
<td></td>
</tr>
<tr>
<td>Johns, B ............................................................... 118</td>
<td></td>
</tr>
<tr>
<td>Johnson, B ............................................................ 77</td>
<td></td>
</tr>
<tr>
<td>Johnson, J ............................................................. 23</td>
<td></td>
</tr>
<tr>
<td>Johnson, K ............................................................ 161</td>
<td></td>
</tr>
<tr>
<td>Johnson, S ............................................................ 157</td>
<td></td>
</tr>
<tr>
<td>Jordan, R ............................................................. 22, 222</td>
<td></td>
</tr>
<tr>
<td>Joseph, S .............................................................. 28, 102</td>
<td></td>
</tr>
<tr>
<td>Julander, J ............................................................. 89, 137</td>
<td></td>
</tr>
<tr>
<td>Jung, S ................................................................. 45</td>
<td></td>
</tr>
<tr>
<td>Jung, Y ................................................................. 127</td>
<td></td>
</tr>
<tr>
<td>Keilholz, L ............................................................. 152</td>
<td></td>
</tr>
<tr>
<td>Khan, L ................................................................. 130</td>
<td></td>
</tr>
<tr>
<td>Khan, M ................................................................. 126</td>
<td></td>
</tr>
<tr>
<td>Kiani, N ................................................................. 206</td>
<td></td>
</tr>
<tr>
<td>Kiarie, J ................................................................. 23</td>
<td></td>
</tr>
<tr>
<td>Kim, C ................................................................. 56, 127</td>
<td></td>
</tr>
<tr>
<td>Kim, M ................................................................. 56, 127</td>
<td></td>
</tr>
<tr>
<td>Kim, S ................................................................. 56</td>
<td></td>
</tr>
<tr>
<td>Kincer, L ............................................................... 28</td>
<td></td>
</tr>
<tr>
<td>Kinney, B .............................................................. 40</td>
<td></td>
</tr>
<tr>
<td>Kirby, K ................................................................. 128</td>
<td></td>
</tr>
<tr>
<td>Klasse, P ............................................................... 58</td>
<td></td>
</tr>
<tr>
<td>Koether, K ............................................................. 129</td>
<td></td>
</tr>
<tr>
<td>Kollanur, D ............................................................ 212</td>
<td></td>
</tr>
<tr>
<td>Komanic-Meredith, G ................................................. 93</td>
<td></td>
</tr>
<tr>
<td>Konreddy, A ............................................................ 70</td>
<td></td>
</tr>
<tr>
<td>Koopmans, M .......................................................... 30</td>
<td></td>
</tr>
<tr>
<td>Korba, B ............................................................... 21, 72, 123, 172</td>
<td></td>
</tr>
<tr>
<td>Koster, A ............................................................... 220</td>
<td></td>
</tr>
<tr>
<td>Kramps, T ............................................................. 144</td>
<td></td>
</tr>
<tr>
<td>Kraus, G ............................................................... 44</td>
<td></td>
</tr>
<tr>
<td>Krecmerova, M ......................................................... 57</td>
<td></td>
</tr>
<tr>
<td>Krumpe, L .............................................................. 145</td>
<td></td>
</tr>
<tr>
<td>Kumar, B ............................................................... 126</td>
<td></td>
</tr>
<tr>
<td>Kumar, R ............................................................... 130</td>
<td></td>
</tr>
<tr>
<td>Kumari, R .............................................................. 130</td>
<td></td>
</tr>
<tr>
<td>Kuzmin, V .............................................................. 62</td>
<td></td>
</tr>
<tr>
<td>Kwan, D ............................................................... 77</td>
<td></td>
</tr>
<tr>
<td><strong>K</strong></td>
<td></td>
</tr>
<tr>
<td>Kadow, J ............................................................... 91</td>
<td></td>
</tr>
<tr>
<td>Kaiser, S .............................................................. 104</td>
<td></td>
</tr>
<tr>
<td>Kalen, K ............................................................... 144</td>
<td></td>
</tr>
<tr>
<td>Kang, C ............................................................... 56</td>
<td></td>
</tr>
<tr>
<td>Kannan, M ............................................................ 99</td>
<td></td>
</tr>
<tr>
<td>Kapteyn, S ............................................................ 214</td>
<td></td>
</tr>
<tr>
<td>Karabiber, F ........................................................... 46</td>
<td></td>
</tr>
<tr>
<td>Karampuri, S .......................................................... 27</td>
<td></td>
</tr>
<tr>
<td>Karla, B ............................................................... 111</td>
<td></td>
</tr>
<tr>
<td>Kashuba, Pharm.D., A ............................................... 98</td>
<td></td>
</tr>
<tr>
<td>Kathur Reddy, M ...................................................... 148</td>
<td></td>
</tr>
<tr>
<td>Katz, Ph.D., D ........................................................ 96</td>
<td></td>
</tr>
<tr>
<td>Katz, D ................................................................. 48</td>
<td></td>
</tr>
<tr>
<td>Kaushik-Basu, N ....................................................... 66</td>
<td></td>
</tr>
<tr>
<td>Keicher, J ............................................................. 142</td>
<td></td>
</tr>
<tr>
<td><strong>L</strong></td>
<td></td>
</tr>
<tr>
<td>Li, C ................................................................. 216</td>
<td></td>
</tr>
<tr>
<td>Lozitsky, V ............................................................ 59</td>
<td></td>
</tr>
<tr>
<td>La Regina, G .......................................................... 42, 66</td>
<td></td>
</tr>
<tr>
<td>Laidlaw, M ............................................................ 41</td>
<td></td>
</tr>
<tr>
<td>Lambert, A ............................................................ 74</td>
<td></td>
</tr>
<tr>
<td>Lampert, B ........................................................... 152</td>
<td></td>
</tr>
<tr>
<td>Landesman, Y ........................................................ 157</td>
<td></td>
</tr>
<tr>
<td>Lange, S ............................................................... 146</td>
<td></td>
</tr>
<tr>
<td>Lanier, R .............................................................. 67, 69, 152</td>
<td></td>
</tr>
<tr>
<td>Lanke, K ............................................................... 76, 207</td>
<td></td>
</tr>
<tr>
<td>Larson, D ............................................................. 159</td>
<td></td>
</tr>
<tr>
<td>LeBranche, C ........................................................ 102</td>
<td></td>
</tr>
<tr>
<td>Leeman, D ............................................................. 58</td>
<td></td>
</tr>
<tr>
<td>Lecavalier, D .......................................................... 147</td>
<td></td>
</tr>
<tr>
<td>Lee, C ................................................................. 127</td>
<td></td>
</tr>
<tr>
<td>Lee, J ................................................................. 56</td>
<td></td>
</tr>
<tr>
<td>Lefebvre, D ........................................................... 212</td>
<td></td>
</tr>
</tbody>
</table>
## Author Index

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lehmann, K</td>
<td>217</td>
</tr>
<tr>
<td>Lemm, J</td>
<td>91</td>
</tr>
<tr>
<td>Levitt, B</td>
<td>51</td>
</tr>
<tr>
<td>Levrero, Ph.D., M</td>
<td>11</td>
</tr>
<tr>
<td>Leyssen, Ph.D., P</td>
<td>205</td>
</tr>
<tr>
<td>Leyssen, P</td>
<td>26, 76, 82, 88, 214</td>
</tr>
<tr>
<td>Li, C</td>
<td>214</td>
</tr>
<tr>
<td>Li, Q</td>
<td>20</td>
</tr>
<tr>
<td>Li, X</td>
<td>149</td>
</tr>
<tr>
<td>Lichière, J</td>
<td>216</td>
</tr>
<tr>
<td>Liljegqvist, J</td>
<td>146</td>
</tr>
<tr>
<td>Limpens, R</td>
<td>220</td>
</tr>
<tr>
<td>Lin, J</td>
<td>103</td>
</tr>
<tr>
<td>Lin, R</td>
<td>95</td>
</tr>
<tr>
<td>Lind, K</td>
<td>142</td>
</tr>
<tr>
<td>Liu, D</td>
<td>116, 131</td>
</tr>
<tr>
<td>Liu, F</td>
<td>119</td>
</tr>
<tr>
<td>Lo, M</td>
<td>19</td>
</tr>
<tr>
<td>Lok, M.D., A</td>
<td>7</td>
</tr>
<tr>
<td>Lomonosova, E</td>
<td>75</td>
</tr>
<tr>
<td>Lorenzo, M</td>
<td>24</td>
</tr>
<tr>
<td>Lozitsky, V</td>
<td>33</td>
</tr>
<tr>
<td>Lozytskyi, V</td>
<td>73</td>
</tr>
<tr>
<td>Lu, H</td>
<td>91</td>
</tr>
<tr>
<td>Lu, R</td>
<td>40</td>
</tr>
<tr>
<td>Ludwig, S</td>
<td>120, 129</td>
</tr>
<tr>
<td>Lundin, A</td>
<td>90</td>
</tr>
<tr>
<td>Lustig, W</td>
<td>48</td>
</tr>
<tr>
<td>Luthra, K</td>
<td>130</td>
</tr>
<tr>
<td>Matheka, D</td>
<td>132</td>
</tr>
<tr>
<td>Matisyewska, N</td>
<td>133</td>
</tr>
<tr>
<td>Maurizio, P</td>
<td>134</td>
</tr>
<tr>
<td>Mayes, B</td>
<td>43</td>
</tr>
<tr>
<td>McCutcheon, K</td>
<td>22</td>
</tr>
<tr>
<td>McLaughlin, R</td>
<td>161</td>
</tr>
<tr>
<td>McMahon, J</td>
<td>145</td>
</tr>
<tr>
<td>Meanwell, Ph.D., N</td>
<td>1</td>
</tr>
<tr>
<td>Meier, C</td>
<td>39, 63, 84</td>
</tr>
<tr>
<td>Melia, C</td>
<td>220</td>
</tr>
<tr>
<td>Menne, Ph.D., S</td>
<td>14</td>
</tr>
<tr>
<td>Mesecar, A</td>
<td>176</td>
</tr>
<tr>
<td>Mesquita, P</td>
<td>34, 158</td>
</tr>
<tr>
<td>Meyer, H</td>
<td>24</td>
</tr>
<tr>
<td>Meyers, M</td>
<td>75</td>
</tr>
<tr>
<td>Miller, D</td>
<td>147</td>
</tr>
<tr>
<td>Miller, S</td>
<td>77, 160</td>
</tr>
<tr>
<td>Miranda Saksena, M</td>
<td>156</td>
</tr>
<tr>
<td>Miranda, S</td>
<td>110</td>
</tr>
<tr>
<td>Mishra, R</td>
<td>72</td>
</tr>
<tr>
<td>Mitchell, D</td>
<td>26</td>
</tr>
<tr>
<td>Miti, A</td>
<td>138</td>
</tr>
<tr>
<td>Mitsuya, H</td>
<td>83</td>
</tr>
<tr>
<td>Mobeyen, H</td>
<td>61</td>
</tr>
<tr>
<td>Moffat, J</td>
<td>116, 115</td>
</tr>
<tr>
<td>Mohmed, S</td>
<td>74</td>
</tr>
<tr>
<td>Moir, D</td>
<td>93</td>
</tr>
<tr>
<td>Mokaya, J</td>
<td>132</td>
</tr>
<tr>
<td>Monetefiori, D</td>
<td>102</td>
</tr>
<tr>
<td>Moore, S</td>
<td>43</td>
</tr>
<tr>
<td>Moran, E</td>
<td>75</td>
</tr>
<tr>
<td>Moreno, A</td>
<td>153</td>
</tr>
<tr>
<td>Morin, B</td>
<td>136</td>
</tr>
<tr>
<td>Morrey, Ph.D., J</td>
<td>13</td>
</tr>
<tr>
<td>Morrey, J</td>
<td>123, 137</td>
</tr>
<tr>
<td>Morris, L</td>
<td>23</td>
</tr>
<tr>
<td>Mostafavi, H</td>
<td>60, 61</td>
</tr>
<tr>
<td>Mote, A</td>
<td>110</td>
</tr>
<tr>
<td>Motter, N</td>
<td>137</td>
</tr>
<tr>
<td>Moulaei, T</td>
<td>35</td>
</tr>
<tr>
<td>Moussa, A</td>
<td>43</td>
</tr>
<tr>
<td>Mudryk, L</td>
<td>33</td>
</tr>
<tr>
<td>Munshi, S</td>
<td>138, 139</td>
</tr>
<tr>
<td>Murao, L</td>
<td>212</td>
</tr>
<tr>
<td>Muratov, E</td>
<td>62</td>
</tr>
<tr>
<td>Murray, E</td>
<td>58</td>
</tr>
<tr>
<td>Mwimanzi, P</td>
<td>160</td>
</tr>
</tbody>
</table>

## N

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na, C</td>
<td>121</td>
</tr>
<tr>
<td>Nack, T</td>
<td>63</td>
</tr>
<tr>
<td>Naesens, L</td>
<td>94</td>
</tr>
<tr>
<td>Ndongwe, T</td>
<td>131, 140</td>
</tr>
<tr>
<td>Neamati, N</td>
<td>151</td>
</tr>
<tr>
<td>Nebane, N</td>
<td>87</td>
</tr>
<tr>
<td>Nesterova, N</td>
<td>31</td>
</tr>
<tr>
<td>Neyts, J</td>
<td>26, 38, 66, 76, 78, 82, 88, 164, 173, 181, 218, 212, 214, 219, 221</td>
</tr>
<tr>
<td>Nguyen, M</td>
<td>110</td>
</tr>
<tr>
<td>Nichol, S</td>
<td>19, 202</td>
</tr>
<tr>
<td>Nikolova, I</td>
<td>155</td>
</tr>
<tr>
<td>Nordhoff, C</td>
<td>129</td>
</tr>
<tr>
<td>Nugent, S</td>
<td>32, 48</td>
</tr>
</tbody>
</table>

## O

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Keefe, Ph.D., B</td>
<td>2</td>
</tr>
<tr>
<td>O’Keefe, B</td>
<td>145</td>
</tr>
<tr>
<td>Obolenskaja, M</td>
<td>73</td>
</tr>
<tr>
<td>Ognichenko, L</td>
<td>62</td>
</tr>
<tr>
<td>Ojha, D</td>
<td>27</td>
</tr>
<tr>
<td>Okamoto, M</td>
<td>141, 163</td>
</tr>
<tr>
<td>Olagnier, D</td>
<td>95</td>
</tr>
<tr>
<td>Otmar, M</td>
<td>57</td>
</tr>
<tr>
<td>Otsuka, Y</td>
<td>210</td>
</tr>
<tr>
<td>Oyero, O</td>
<td>178</td>
</tr>
<tr>
<td>O’Keefe, B</td>
<td>35</td>
</tr>
</tbody>
</table>

## P

<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Padmanabhan, S</td>
<td>21, 123, 172</td>
</tr>
<tr>
<td>Pan, Q</td>
<td>108</td>
</tr>
<tr>
<td>Pandey, R</td>
<td>21, 123, 172</td>
</tr>
<tr>
<td>Pandi, T</td>
<td>150</td>
</tr>
<tr>
<td>Panecouque, C</td>
<td>150</td>
</tr>
<tr>
<td>Parks, D</td>
<td>142, 118</td>
</tr>
<tr>
<td>Parniak, M</td>
<td>128</td>
</tr>
<tr>
<td>Parsley, T</td>
<td>64</td>
</tr>
<tr>
<td>Pathak, K</td>
<td>65</td>
</tr>
<tr>
<td>Pathak, V</td>
<td>140</td>
</tr>
<tr>
<td>Paul, D</td>
<td>222</td>
</tr>
<tr>
<td>Pauls, E</td>
<td>143</td>
</tr>
<tr>
<td>Pearlman, A</td>
<td>166</td>
</tr>
<tr>
<td>Pelliccia, S</td>
<td>66</td>
</tr>
<tr>
<td>Author Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Tabassum, S</td>
<td>138, 139</td>
</tr>
<tr>
<td>Taghizadeh, Z</td>
<td>61</td>
</tr>
<tr>
<td>Talaei Zanjani, N</td>
<td>156</td>
</tr>
<tr>
<td>Tamir, S</td>
<td>157</td>
</tr>
<tr>
<td>Tanaka, Y</td>
<td>72</td>
</tr>
<tr>
<td>Taneva, E</td>
<td>158</td>
</tr>
<tr>
<td>Tang, J</td>
<td>128</td>
</tr>
<tr>
<td>Tang, X</td>
<td>17</td>
</tr>
<tr>
<td>Tang, Y</td>
<td>149</td>
</tr>
<tr>
<td>Tarbet, B</td>
<td>159</td>
</tr>
<tr>
<td>Tas, A</td>
<td>88, 209</td>
</tr>
<tr>
<td>Tavis, J</td>
<td>75</td>
</tr>
<tr>
<td>Thibaut, H</td>
<td>76, 78, 207</td>
</tr>
<tr>
<td>Thiel, V</td>
<td>90</td>
</tr>
<tr>
<td>Thierry, S</td>
<td>114</td>
</tr>
<tr>
<td>Thomson, M</td>
<td>142, 165</td>
</tr>
<tr>
<td>Tietjen, I</td>
<td>77, 160</td>
</tr>
<tr>
<td>Tijsma, A</td>
<td>78, 221</td>
</tr>
<tr>
<td>Tiong-Yip, C</td>
<td>107, 161</td>
</tr>
<tr>
<td>Tiwari, A</td>
<td>130</td>
</tr>
<tr>
<td>Toncheva, D</td>
<td>162</td>
</tr>
<tr>
<td>Topalis, D</td>
<td>24, 94</td>
</tr>
<tr>
<td>Toyama, M</td>
<td>70, 141, 163</td>
</tr>
<tr>
<td>Tramontano, E</td>
<td>114</td>
</tr>
<tr>
<td>Tripp, R</td>
<td>157</td>
</tr>
<tr>
<td>Trist, I</td>
<td>214, 222</td>
</tr>
<tr>
<td>Trybala, E</td>
<td>90, 146</td>
</tr>
<tr>
<td>Tsyrkunov, V</td>
<td>133</td>
</tr>
<tr>
<td>Ulferts, Ph.D., R</td>
<td>205</td>
</tr>
<tr>
<td>Urban, M.D., S</td>
<td>8</td>
</tr>
<tr>
<td>Valdar, W</td>
<td>134</td>
</tr>
<tr>
<td>Valtchev, P</td>
<td>156</td>
</tr>
<tr>
<td>van den Hoogen, B</td>
<td>38</td>
</tr>
<tr>
<td>van der Avoort, H</td>
<td>30</td>
</tr>
<tr>
<td>van der Linden, L</td>
<td>164</td>
</tr>
<tr>
<td>van der Poel, H</td>
<td>26</td>
</tr>
<tr>
<td>van der Schaar, H</td>
<td>26, 76, 207, 215, 220</td>
</tr>
<tr>
<td>van Hemert, M</td>
<td>38, 88, 95, 209</td>
</tr>
<tr>
<td>Van Kuppeveld, F</td>
<td>26</td>
</tr>
<tr>
<td>van Kuppeveld, Ph.D., F</td>
<td>203, 204</td>
</tr>
<tr>
<td>van Kuppeveld, F</td>
<td>76, 164, 211, 215, 220</td>
</tr>
<tr>
<td>Van Loock, M</td>
<td>218</td>
</tr>
<tr>
<td>Van Puyenbroeck, V</td>
<td>29, 37</td>
</tr>
<tr>
<td>van Veelen, P</td>
<td>217</td>
</tr>
<tr>
<td>Varlamova, E</td>
<td>62</td>
</tr>
<tr>
<td>Vasylinekenko, O</td>
<td>73</td>
</tr>
<tr>
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</tr>
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<td>29, 37</td>
</tr>
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</tr>
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<td>164</td>
</tr>
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<td>Vogel, A</td>
<td>144</td>
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</tr>
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<td>53, 177</td>
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</tr>
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<td>53</td>
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<td>83</td>
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<td>12</td>
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<td>133</td>
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<td>58</td>
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<tr>
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</tr>
</tbody>
</table>

Program and Abstracts of the 27th International Conference on Antiviral Research (ICAR)
The 28th International Conference on Antiviral Research (ICAR), hosted by the International Society for Antiviral Research (ISAR), will take place at the Parco dei Principi Hotel, Rome, Italy. The conference will begin on Monday, May 11th, 2015, and will conclude on Friday, May 15th, 2015.

ICAR provides an interdisciplinary forum of interest to chemists, biologists, and clinicians involved in antiviral research. In 2015, scientists worldwide working in the areas of basic, applied, and clinical research meet in a collaborative and collegial atmosphere to review recent developments in all areas of antiviral drug discovery and development.

Specific topics to be covered in the scientific program include:
- Medicinal chemistry
- Virus replication
- Host cell-virus interactions
- Virus latency
- New target identification
- Biochemistry and mechanism of action
- Mechanisms of viral drug resistance
- Assay development
- In vitro evaluation
- Animal models
- Pharmacokinetics
- Toxicology
- Clinical trials

Rome, the capital of Italy, has a long tradition of scientific innovation, making it the ideal location for ICAR. By meeting in the heart of the city, ICAR delegates will be able to take advantage of the atmosphere and energy of this unique city ensuring the best of both worlds, a world class destination and a rigorous scientific content.