Program and Abstracts of the Twenty-Fifth International Conference on Antiviral Research (ICAR)

Sapporo, Japan, April 16 – 19, 2012

International Society For Antiviral Research

抗ウイルス療法研究会（JAAT）
Japanese association for antiviral Therapy
Program and Abstracts

Twenty-Fifth International Conference on
Antiviral Research

Co-Sponsored by the
International Society for Antiviral Research (ISAR)
and the
Japanese Association for Antiviral Therapy (JAAT)

Hotel Royton-Sapporo
Sapporo, Japan
April 16 – April 19, 2012
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Organization
International Society for Antiviral Research
and
Twenty-Fifth International Conference on Antiviral Research

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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 25th International Conference on Antiviral Research

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Additional Support Provided by:
Office of AIDS Research, National Institutes of Health, Bethesda, MD, USA
Japan Foundation for AIDS Prevention (JFAP), Tokyo, Japan
City of Sapporo, Sapporo, Japan

Program Flash Drives provided by ImQuest BioSciences, Inc., Frederick, MD, USA
KEYNOTE ADDRESS
“Structure-Guided Development of AIDS Therapeutics: Successes, Challenges, and Opportunities”
Hiroaki Mitsuya, M.D., Ph.D.
Monday April 16, 2012
4:45 – 5:45 PM

25th ANNIVERSARY LECTURE
“Successes and Failures in Antiviral Drug Development: A Personal Account”
Erik De Clercq, M.D., Ph.D.
Monday April 16, 2012
5:45 – 6:30 PM

MINI-SYMPOSIA
"Therapy of Infections Endemic to Japan and Asia”
Tuesday, April 17, 2012
8:00 AM – 12:00 PM

“Clinical Update on Antiviral Drugs”
Wednesday, April 18, 2012
1:00 – 4:00 PM

“Building a Better Clinical Candidate: Issues, Strategies, and Tools
Thursday, April 19, 2012
8:00 AM – 12:00 PM

SOCIAL EVENTS
Opening Reception
with light hors d’oeuvres
Monday, April 16, 2012
6:30 – 8:30 PM

Conference Banquet
Thursday April 19, 2012
Reception 7:15 PM
Dinner 7:45 – 10:00 PM

ICAR Career Forum
Wednesday, May 18, 2012
6:30 to 8:30 pm
Pearl Hall on 20th Floor

All Scientific and Social Events will be held in the Hotel Royton Sapporo,
Sapporo, Japan
Final Program

Twenty-Fifth International Conference on Antiviral Research

Sponsored by the International Society for Antiviral Research and the Japanese Association for Antiviral Therapy

Hotel Royton Sapporo
Sapporo, Japan

April 16 – April 19, 2012
Monday, April 16, 2012

Interactive Workshop: Drug Discovery and Development 101
Chair(s): Joseph Colacino, Ph.D. and Phillip Furman, Ph.D.
Royton Hall AB, 3rd Floor
02:00 PM - 04:00 PM

14:00 1. High Throughput Screening and Drug Development.
      Raj Kalker, Ph.D.
      Vertex Pharmaceuticals, USA

      Sina Bavari, Ph.D.
      United States Army Medical Research Institute of Infectious Diseases, USA

Opening Greetings and Welcome to Sapporo
Royton Hall AB, 3rd Floor
04:30 PM - 04:45 PM

16:30 Welcome to the 25th ICAR: Joseph Colacino, Ph.D., President, ISAR
      Welcome to Sapporo: Masanori Baba, M.D., Ph.D., Local Host
      Introduction of the Keynote Speaker: Phillip Furman, Ph.D., President-Elect, ISAR.

Keynote Address
Chair(s): Phillip Furman, Ph.D.
Royton Hall AB, 3rd Floor
04:45 PM - 05:45 PM

      Hiroaki Mitsuya, M.D., Ph.D.
      National Cancer Institute, USA and Kumamoto University School of Medicine, Japan

25th Anniversary Lecture
Royton Hall AB, 3rd Floor
05:45 PM - 06:30 PM

      Erik DeClerq, M.D., Ph.D.
      Rega Institute for Medical Research, Belgium
Opening Reception
Royton Hall Foyer, 3rd Fl
06:30 PM - 08:30 PM

Tuesday, April 17, 2012

Oral Session 1: Mini-Symposium - Therapy of Viral Infections Endemic to Japan and Asia
Chair(s): Masanori Baba, M.D., Ph.D., Hiroaki Mitsuya, M.D., Ph.D., and Robert W. Buckheit, Jr., Ph.D.
Royton Hall AB, 3rd Floor
08:00 AM - 12:00 PM

08:00  5. Therapy of Japanese Encephalitis Virus.
           Kouichi Morita, Ph.D.
           Nagasaki University, Japan

08:30  6. ATL-Like Phenotype in HTLV-1 Infected Humanized Mouse Model.
           Jun-ichi Fujisawa, Ph.D.
           Kansai Medical University, Japan

09:00  7. Therapy of Influenza Virus.
           Simon Tucker, Ph.D.
           Biota, New Zealand

09:30  Break.

10:00  8. Hepatitis C Virus Replication Models and Anti-Viral Development.
           Takaji Wakita, Ph.D.
           NIH, Japan

           Pei Yong Shi, Ph.D.
           Novartis, Singapore

11:00  10. Hepatitis B: Is a Cure Possible, Is it Necessary?
           Timothy Block, Ph.D.
           Hepatitis B Foundation and Drexel University, USA

Gertrude Elion Award Lecture
Royton Hall AB, 3rd Floor
01:30 PM - 02:15 PM

13:30  Presentation of the Gertrude Elion Award: Joseph Colacino, Ph.D., President, ISAR.

           Karl Hostetter, M.D.
           University of California at San Diego, USA
CK Chu¹, M Sugiyama², S Chavre¹, US Singh¹, RK Rawal¹, R Govindarajan¹, B. Korba³, Y Tanaka²  
¹University of Georgia, Athens, United States, ²Nagoya City University, Nagoya, Japan, ³University of Georgia, Athens, United States, ⁴University of Georgia, Athens, United States, ⁵University of Georgia, Athens, United States, ⁶University of Georgia, Athens, United States, ⁷Georgetown University, Washington, DC, United States, ⁸Nagoya City University, Nagoya, Japan

Changhua Ji¹, Sastry K. Seetharama², Georg Tiefenthaler³, Han Ma¹, Stefan Ries², Klaus Klumpp¹, Erhard Kopetzki², Antonio Bertoletti²  
¹Roche Virology Discovery, Nutley, NJ, United States, ²SICS, A STAR, Singapore, Singapore, ³Roche Large Molecule Research, Penzberg, Germany

14:45 14. A Serum Factor Is Critically Required for Efficient Synthesis of Hepadnaviral cccDNA.  
Yong-Yuan Zhang, Robert W Buckheit, Jr.  
ImQuest BioSciences, Inc., Frederick, Maryland, United States

15:00 15. Human Pluripotent Stem Cell Derived Hepatocyte Progeny Support Complete Replication of Hepatitis C Virus.  
P. Roelandt¹,², J. Paeshuyse³, S. Obeid³, J. Vanhove¹, Y. Nahmias⁴, F. Nevens², J. Neyts³, C.M. Verfaillie¹  
¹Interdepartmental Stem Cell Institute, KU Leuven, Leuven, Belgium, ²Hepatology Department, University Hospitals Leuven, Leuven, Belgium, ³Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ⁴Center for Bioengineering, Hebrew University of Jerusalem, Jerusalem, Israel

15:15 16. Discovery of Novel HCV Inhibitors Targeting the Viral NS4B.  
Zhengxian Gu, Nanjing Zhang, Jason Graci, Steve Jung, Gary Karp, Neil Almstead, Joseph Colacino  
PTC Therapeutics, Inc., South Plainfield, NJ, United States

15:30 17. Discovery of a Novel Non-Nucleoside Inhibitor of HCV NS5B Which Possesses Broad Genotypic Potency and an Attractive Pre-Clinical Profile.  
Steve Ludmerer, Fangbiao Li, Peter Meinke, Carmela Molinaro, David B. Olsen, James Ormes, Jin Wu, Casey McComas  
Merck & Co., Whitehouse Station, NJ, United States

15:45 18. Metabolic Activation of the Anti-Hepatitis C Virus Nucleotide Prodrug, PSI-352938.  
Congrong Niu, Tatiana Tolstykh, Haiying Bao, Angela M Lam, Shalini Bansal,
Michael J Sofia, Phillip A Furman, Eisuke Murakami
Pharmasset, Inc., Princeton, NJ, United States

16:00 19. Identification of a Novel Resistance Mutation for Hepatitis C Virus Benzimidazole Inhibitor JT-16.
Leen Delang, Mathy Froeyen, Johan Neyts
Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Yutaka Takebe1, Carrie J. Saucedo2, Garry Lund3, Rie Uenishi1, Norman Knetman4, Takaji Wakita1, James B. McMahon4, Barry R. O'Keefe4
1National Institute of Infectious Diseases, Shinjuku, Tokyo, Japan, 2SAIC-Frederick, Frederick, Maryland, United States, 3KMT Hepatech, Edmonton, Alberta, Canada, 4National Cancer Institute, Frederick, Maryland, United States

Wednesday, April 18, 2012

William Prusoff Young Investigator Award
Royton Hall AB, 3rd Floor
08:00 AM - 08:45 AM

Presentation of the William Prusoff Young Investigator Award: Joseph Colacino, Ph.D., President, ISAR.

21. HBV & HCV: Parallels, Contrasts and Future Directions for Therapy.
William Delaney, Ph.D.
Gilead Sciences, USA

Oral Session 3: Respiratory and Emerging Infections
Chair(s): Simon Tucker, Ph.D. and Johan Neyts, Ph.D.
Royton Hall AB, 3rd Floor
08:45 AM - 11:45 AM

08:45 22. Novel Fusion Inhibitors of Influenza Virus.
Ming Luo1, Shihong Qiu1, Guoxin Wang2, Michael J. Rowse1, Jun Tsao1, Todd J. Green1, Zhen Yang3
1The University of Alabama at Birmingham, Birmingham, AL, United States, 2Fuzians Biomedicals, Ltd., Shenzhen, China, 3Shenzhen Graduate School of Peking University, Shenzhen, China

09:00 23. Discovery and Development of Orally Active Antivirals for the Treatment of RSV: Identification of a Second Generation Candidate.
Simon Tucker, David Bourke, Alistair Draffan, Jennifer Fenner, Jega Iswaran, John Lambert, Penny Mayes, Gary Pitt
Biota Scientific Management, Pty, Ltd, Melbourne, Victoria, Australia

Bart Tarbet, Brett Hurst, Bentley Anderson, Tyler McLean, John Morrey
09:30  Break.

10:00  25. Therapeutic Efficacy of ST-246 in Cynomolgous Macaques Challenged with Monkeypox Virus Via Aerosol.  
Lovelace Respiratory Research Institute, Albuquerque, NM, United States

H.-J. Thibaut1, L. van der Linden2, B. Thys3, A. De Palma1, M.J. Pérez-Pérez4, B. Rombaut2, F. Van Kuppeveld2, J. Neyts1  
1Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, 2Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, 3Vrije Universiteit Brussel, Brussel, Belgium, 4Instituto de Química Instituto de Química Médica, Madrid, Spain

10:30  27. A New Mouse Model of Chikungunya Virus with Utility in Antiviral Studies.  
Justin Julander1, Ashley Dagley1, Jane Ennis2, John Morrey1, Jeff Turner2  
1Utah State University, Logan, UT, United States, 2Defyrus Inc., Toronto, ON, Canada

10:45  28. Inhibitor of Human Coronavirus 229E Infectivity Targeting Viral Non-Structural Protein 6 Involved in Modulation of Cellular Membranes.  
Anna Lundin1, Tomas Bergström1, Nina Kann2, Beata Adamiak1, Charles Hannoun1, Edward Trybala1  
1Department of Clinical Virology, University of Gothenburg, Gothenburg, Sweden, 2Organic Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

11:00  29. The Rafis AUY11 and DUY11 Inhibit Infectivity of Unrelated Enveloped Viruses by Preventing Fusion of Viral and Cellular Membranes.  
Che C. Colpitts1, Alexey V. Ustinov2, Vladimir A. Korshun2, Luis M. Schang1  
1University of Alberta, Edmonton, Canada, 2Russian Academy of Sciences, Moscow, Russia

11:15  30. Inhibition of Cellular P38 Map Kinase Impairs Influenza Virus Induced Primary and Secondary Host Gene Responses and Protects Mice from Lethal H5N1 Infection.  
Yvonne Boergeling1, Mirco Schmolke1,3, Dorothee Viemann2,4, Johannes Roth2, Stephan Ludwig1  
1University of Muenster, Institute of Molecular Virology, Muenster, NRW, Germany, 2University of Muenster, Institute of Immunology, Muenster, NRW, Germany, 32present address: Mount Sinai School of Medicine, Department of Microbiology, New York, NY, United States, 4present address: Department of Pediatric Pulmonology, Allergology and Neonatology, Hannover, Germany

11:30  31. 3',5'-Di-O-Trityluridine Inhibits Dengue and Yellow Fever Virus Replication In Vitro, Specifically Targeting the Initiation Process of the Viral RNA Polymerization.  
Tine De Burghgraeve1, Suzanne Kaptein1, Barbara Selisko2, Mathy Froeyen1, Michael Jacobs3, Bruno Canard2, Arthur Van Aerschot1, Johan Neyts1
Clinical Symposium
Royton Hall AB, 3rd Floor
01:00 PM - 03:00 PM

Oral Session 4: Mini-Symposium: Clinical Development of Antiviral Agents
Chair(s): Phillip Furman, Ph.D., USA
Royton Hall AB, 3rd Floor
03:00 PM - 04:30 PM

15:00 ISAR Business Meeting

15:30 32. Seroprevalence of Dengue Virus Specific IgG Antibodies Among Apparently Healthy Individuals in Ibadan, South-Western, Nigeria.
Olufunmilayo G. Oyero
The Polytechnic, Ibadan, Oyo, Nigeria

15:45 33. Resistance Genotypes in CMX001-201 Clinical Trial for CMV Prophylaxis.
Scott Foster, Alice Robertson, George Painter, Susan Godkin, Wendy Painter, Randall Lanier
Chimerix, Inc., Durham, NC, United States

16:00 34. Genotypic Characterization of HCV Variants from the Proof of Concept Study of Miravirsen (MIR), an Oligonucleotide Targeting miR-122, in Treatment Naïve Patients with Genotype 1 (Gt1) Chronic HCV Infection.
Amy K Patick¹, Alice Chen¹, Leen-Jan van Doorn², Eva M van der Veer², Karin Zeh³, Anneke K Raney¹, Michael R Hodges¹
¹Santaris Pharma, San Diego, CA, United States, ²DDL Diagnostic Laboratory, Voorburg, the Netherlands,

16:15 35. Multiple Development Pathways of Pyrimidinediones as Topical Microbicides to Prevent the Transmission of HIV-1.
Karen W Buckheit¹, Anthony Ham¹, Patrick Kiser³, Charlene Dezzutti³, Robert W Buckheit, Jr¹
¹ImQuest BioSciences, Inc., Frederick, Maryland, United States, ²University of Utah, Salt Lake City, Utah, United States, ³Magee Womens Research Institute, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Thursda, April 19, 2012

Oral Session 5: Mini-Symposium - Building a Better Clinical Candidate: Issues, Strategies, and Tools
Chair(s): Michael Sofia, Ph.D., Pharmasset
Royton Hall AB, 3rd Floor
08:00 AM - 12:00 PM
08:00 36. Role of Prodrug Design in Drug Discovery and Lead Optimization.
   Reza Oliyai, Ph.D.
   Gilead Sciences, USA

   Robert Zahler, Ph.D.
   PharmD Consulting, USA

09:00 38. The Discovery of Asunaprevir (BMS-650032): a Protease Inhibitor for the Treatment of Hepatitis C Virus.
   Paul Scola, Ph.D.
   Bristol Myers Squibb, USA

09:30 Break.

10:00 39. HCV Protease Inhibitors: Discovery of ACH1625 and ACH2684.
   Avinash Phadke, Ph.D.
   Achillion, USA

10:30 40. IDX184 and Other Nucleotide Prodrug Candidates for the Therapy of HCV.
   Dominique Surleraux, Ph.D.
   Idenix, USA

Oral Session 6: Retroviruses and Herpes Viruses
Chair(s): Jose Este, Ph.D. and Graciela Andrei, Ph.D.
Royton Hall AB, 3rd Floor
01:30 PM - 03:45 PM

   Joachim Hauber
   Heinrich-Pette Institute, Hamburg, Germany

14:00 41. Prodrugs of Acyclic Nucleoside Phosphonates: Novel Approaches.
   Zlatko Janeba
   IOCB AS CR, Prague, Czech Republic

14:15 42. Screening and Synthesis of Deoxyhypusine Synthase Inhibitors Targeting a Cellular Factor Needed in HIV-1 Replication.
   Chris Meier¹, Marcus Schroeder¹, Adrian Kolodzik¹, Marcel Krepstakies², Rolf Hilgenfeld³, Jan van Lunzen⁴, Joachim Hauber³, Matthias Rarey¹
   ¹University of Hamburg, Hamburg, Germany, ²Heinrich Pette Institute, Hamburg, Germany, ³Lübeck University, Lübeck, Germany, ⁴University Medical Center, Hamburg, Germany

14:30 43. A Phenylthiadiazolylideneamine Derivative That Potently Ejects Zinc from Both Retroviral Nucleocapsid Zinc Fingers Inactivates HIV Virions by Destabilizing the Viral Genomic RNA.
Thomas Vercruysse¹, Beata Basta², Nicolas Humbert², Wim Dehaen², Jan Balzarini¹, Christophe Pannecouque¹, Yves Mély², Dirk Daelemans¹
¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²Laboratoire de Biophotonique et Pharmacologie, Université de Strasbourg, Illkirch, France,
³Department of Molecular Design and Synthesis, KU Leuven, Leuven, Belgium

14:45 44. Compensatory Mutations Rescued the Virus Replicative Capacity of VIRIP Resistant and VIR576 Cross-Resistant HIV-1.
Emmanuel Gonzalez, Ester Ballana, Roger Badia, Bonaventura Clotet, Jose A. Este
Iriscâixa, Badalona, Barcelona, Spain

15:00 45. Anti-HIV-1 Gene Therapy Using Autologous CD4+ T Cells Modified with a Retroviral Vector Expressing a Bacterial Endoribonuclease MazF.
Hideto Chono¹, Mika Okamoto², Koichi Inoue¹, Katsuyuki Dodo¹, Hiroshi Tsuda¹, Naoki Saito¹, Masanori Baba², Junichi Mineno¹
¹Center for Cell and Gene Therapy, Takara Bio Inc., Otsu, Shiga, Japan, ²Center for Chronic Viral Diseases, Kagoshima University, Kagoshima, Kagoshima, Japan

15:15 46. Sensitivity of Clinical Isolates to Helicase Primase Inhibitors and No Detection of Resistance Mutations Above Background Frequency.
Hugh J Field¹, Ian Mickleburgh¹, Meei-Li Huang², Laurence Tiley¹, Anna Wald², Helga Ruebsamen-Schaeff³, Holger Zimmermann³, Alexander Birkmann³
¹University of Cambridge, Cambridge, Cambridge, United Kingdom, ²University of Washington, Seattle, Washington, United States, ³AiCuris GmbH & Co, Wuppertal, Germany

15:30 48. Excellent Efficacy and Pharmacokinetics Have Been Demonstrated in Pre-Clinical and Phase I/II Studies by AIC316, a Novel Drug Against Herpes Simplex Virus (HSV) Type 1 and 2.
Alexander Birkmann, David McCormick, Dirk Kropeit, Burkhard Timmler, Susanne Stoelben, Marie Paule Richard, Holger Zimmermann, Helga Ruebsamen-Schaeff
AiCuris GmbH & Co. KG, Wuppertal, Germany

Graciela Andrei, Pierre Fiten, Ghislain Opdenakker, Robert Snoeck
Rega Institute for Medical Research. KU Leuven, Leuven, Belgium

Late Breaker and Shotgun Poster Presentations
Chair(s): Robert W. Buckheit, Jr., Ph.D. and Mark Prichard, Ph.D.
Royton Hall AB, 3rd Floor
04:00 PM - 05:00 PM

ICAR Banquet Reception
Royton Hall Foyer, 3rd Floor
Poster Session 1: Retroviruses, Hepatitis Virus, Respiratory Viruses, Emerging Viruses and Antiviral Methods
Royton Hall CD, 3rd Floor
04:15 PM - 06:15 PM

50. A Novel Peptide Derived from Measles Virus Fusion Protein Inhibits the Replication of Subacute Sclerosing Panencephalitis (SSPE) Virus In Vitro and In Vivo.
Masahiro Watanabe¹, Koichi Hashimoto¹, Yusaku Abe¹, Eiichi Kodama², Ryota Nabika³, Shinya Oishi³, Nobutaka Fujii³, Mitsuaki Hosoya¹
¹Department of Pediatrics, Fukushima Medical University, Fukushima, Fukushima, Japan, ²Division of Emerging Infectious Diseases, Tohoku University School of Medicine, Sendai, Miyagi, Japan, ³Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Kyoto, Japan

51. Generation of Dengue Virus Resistant to Carbohydrate-Binding Agents Results in the Deletion of the Unique N-Glycosylation Sites on the Viral Envelope E-Glycoprotein.
Marijke MF Alen, Kai Dallmeier, Jan Balzarini, Johan Neyts, Dominique Schols
Rega Institute for Medical Research, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

52. Labyrinthopeptins, a New Class of Lantibiotics, Exhibit Potent Anti-Dengue Virus Activity.
Marijke MF Alen¹, Johan Neyts¹, Roderich D Süssmuth², Mark Brönstrup³, Dominique Schols¹
¹Rega Institute for Medical Research, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium, ²Technische Universität Berlin, Fakultät II – Institut für Chemie, Berlin, Germany, ³Sanofi R&D, Industriepark Hoechst, Frankfurt, Germany

Masayuki Amano¹, Yasushi Tojo¹, Manabu Aoki¹, Joseph R. Campbell¹, Chun X. Xu², Kalapala V. Rao³, Arun K. Ghosh², Hiroaki Mitsuya¹³
¹Kumamoto U., Kumamoto, Kumamoto, Japan, ²Purdue U., W Lafayette, IN, United States, ³Exp Retrovirol Sect, NCI, NIH, DHHS, Bethesda, MD, United States

55. Mutations in NS5A-ISDR in Non-Responders of Combination Therapy of HCV 3A Infected Pakistani Patients.
Binish G Arshad1,2, Abida Raza1, Javaid Irfan1, Shahnaz Murtaza1, Samina Shakeel1
1Nuclear Medicine, Oncology and Radiotherapy Institute, Islamabad, Pakistan,
2Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan

56. Inhibition of the HIV-1 Rev-Mediated mRNA Export Pathway by Small Molecules Prevents Viral Replication.
Eline Boons1, Maarten Jacquemyn1, Vincent Sandanayaka2, Sharon Shechter2,
Sharon Shacham2, Michael Kauffman2, Christophe Pannecouque3, Dirk Daelemans1
1Rega Institute, KU Leuven, Leuven, Belgium, 2Karyopharm Therapeutics, Natick, MA, United States

Tracy Hartman, Karen W Buckheit, Robert W Buckheit, Jr
ImQuest BioSciences, Inc., Frederick, Maryland, United States

58. PD 404,182 Is a Virucidal Small Molecule That Disrupts Hepatitis C Virus and Human Immunodeficiency Virus.
Ana M Chamoun1, Karuppiah Chockalingam1, Michael Bobardt2, Rudo Simeon1,
Jinhong Chang3, Philippe Gallay2, Zhilei Chen1
1Texas A&M University, College Station, TX, United States, 2The Scripps Research Institute, La Jolla, CA, United States, 3Drexel Institute for Biotechnology and Virology Research, Doylestown, PA, United States

59. Liver X Receptors Agonists Impede Hepatitis C Virus Infection in an Idol-Dependent Manner.
Xulin C. Chen, Jing Z. Zeng, Yang W. Wu, Qingjiao L. Liao, Lixia L. Li, Xinwen C. Chen
Wuhan Institute of Virology, CAS, Wuhan, Hubei, China

60. A Mechanism of Anti-Influenza A Virus Infection Mediated by Curcumin.
Zih-Yan Chen1, Hsiao-Wei Wen5, Da-Yuan Chen3, Min-Liang Wong2, Wei-Li Hsu3
1Department of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan, 2Department of Food Science and Biotechnology, National Chung Hsing University, Taichung, Taiwan, 3Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung, Taiwan

61. Importance of Crimean Congo Haemorrhagic Fever in Iran as an Emerging Infectious Disease.
Sadegh CHINIKAR1, Sahar KHAKIFIROUZ1, Fereshteh Sadat RASI VARAIE1,
Mahboobeh RAFIGH1, Nareman SHAH HOSSEINI1, Abdolghafar HASAN ZEHI2
1Arboviruses and Viral Haemorrhagic Fevers Laboratory (National Reference Lab), Department of Virology, Pasteur Institute of Iran, Tehran, Tehran, Iran,
Ka Yan Chung1,2, Hongping Dong1, Nahdiyah Abdul Ghafar1, Cheah Chen Seh1, Pei Yong Shi1, Siew Pheng Lim1
1Novartis Institute for Tropical Diseases, Singapore, Singapore, 2Nanyang Technological University, Singapore, Singapore

63. The Green Tea Polyphenol Epigallocatechin Gallate Inhibits Infectivity of Unrelated Enveloped Viruses by Preventing Primary Attachment of Virions to Cells.
Che C. Colpitts1, Sandra Ciesek2, Eike Steinmann2, Luis M. Schang1
1University of Alberta, Edmonton, Canada, 2Twincore, Hannover, Germany

64. Action Mechanism of the Anti-Influenza Virus Active Kampo (Traditional Japanese Herbal) Medicine, Hochuekkitto.
Katsuaki Dan1, Hiroko Akiyoshi2, Kaori Munakata2, Hideki Hasegawa3, Kenji Watanabe2
1Keio University Sch. Med. Collaborative Research Resources, Tokyo, Japan, 2Keio University Sch. Med. Center for Kampo Medicine, Tokyo, Japan, 3National Institute of Infectious Diseases, Tokyo, Japan

65. Antiviral Activity of a Phenolic Dibenylsulfide Against New World Clade B Arenavirus Infections.
Brian B. Gowen, Kie-Hoon Jung, Eric J. Sefing, Min-Hui Wong, Donald F. Smee
Department of Animal, Dairy, and Veterinary Sciences and Institute for Antiviral Research, Utah State University, Logan, Utah, United States

66. Antiviral Activity of Ladania067 an Extract from Ribes nigrum Against Influenza- and Rhinovirus.
Emanuel Haasbach1, Carmen Müller1, Ulrich Wulle1, Christina Ehrhardt2, Stephan Ludwig2, Oliver Planz1
1Department of Immunology, Eberhard Karls University, Tuebingen, Germany, 2Institute of Molecular Virology, ZMBE, University of Muenster, Muenster, Germany

67. The Combination of 4'-Ethynyl-2-Fluoro-2'-Deoxyadenosine with Rilpivirine Shows Synergistic Anti-HIV-1 Activity In Vitro.
Atsuko Hachiya1,2, Bruno Marchand1, Eleftherios Michailidis1, Eiichi N Kodama3, Michael A Parniak4, Hiroaki Mitsuya5,6, Shinichi Oka2, Stefan G Sarafianos1,7
1Departments of Molecular Microbiology & Immunology, University of Missouri School of Medicine, Columbia, MO, United States, 2AIDS Clinical Center, National Center for Global Health and Medicine, Shinjyuku, Tokyo, Japan, 3Division of Emerging Infectious Diseases, Tohoku University School of Medicine, Sendai, Miyagi, Japan, 4Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA, United States, 5Division of Experimental Retrovirology, National Institutes of Health, Bethesda, MD, United States, 6Department of Hematology and Infectious Diseases, Kumamoto University, Kumamoto, Kumamoto, Japan, 7Biochemistry, University of Missouri School of Medicine, Columbia, MO, United States

68. Identification of Small Molecules That Inhibit Tat-Mediated HIV-1 Replication by
Ilona Hauber¹, Helga Hofmann-Sieber¹, Jan Chemnitz¹, Janet Chusainow², Frank Buchholz², Joachim Hauber¹
¹Heinrich Pette Institute - Leibniz Institute for Experimental Virology, Hamburg, 20251, Germany, ²University of Technology Dresden, Department of Medical Systems Biology, Dresden, 01307, Germany

70. Enhancement of HIV Therapeutic Vaccination of Th17 Galt Cell Lines with LAMP, Interleukin-22 and Ganedan BC30 to Control HIV Infection.
B Hearl¹, D Bray², K Benlhassan-Chahour², D Miller³, M Selbovitz⁴, R Moore⁵
¹Immunomic Therapeutics, Bethesda, MD, United States, ²ImmunoClin, Paris, France, ³The AIDS Institute, New York, NY, United States, ⁴NAPWA, Silver Spring, MD, United States, ⁵Associate U.S. Surgeon General (Retired), Rockville, MD, United States

71. Standardized Cell-Based Assays to Evidence Antiviral Effects of New Compounds Against Arbovirus.
Vincent Huyot¹, Laurence Dupuis-Maguiraga², Karen Storck¹, Christine Rogez-Kreuz¹, Roger Le Grand², Pierre Roques², Pascal Clayette¹
¹Neurovirology Department, BERTIN Pharma, CEA, Fontenay aux Roses, France, ²ImmunoVirology Department, imETI/DSV, CEA, Fontenay aux Roses, France

72. Impact of Short Term Anti-Retroviral Therapy (START) on Some Fibrinolytic Markers in Some HIV Infected Adults: Preliminary Findings from the START Study.
Zaccheaus A Jeremiah¹, Yetunde Obazee², Osaro Mgbere³, Ekere J. Essien⁴
¹Niger Delta University, Wilberforce Island, Bayelsa, Nigeria, ²2 General Hospital, Maitama District, Abuja, FCT, Nigeria, ³Department of Health and Human Services, Houston, Texas, United States, ⁴University of Houston, Houston, Texas, United States

73. Diagnosis of Influenza Viruses By Peptide Nucleic Acid-Modified with a Novel Intercalator.
Kunihiro Kaihatsu¹, Shinjiro Sawada¹, Takashi Kanno¹, Shota Nakamura², Takaaki Nakaya³, Teruo Yasunaga³, Nobuo Kato¹
¹The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka, Japan, ²Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan, ³Department of Infectious Diseases, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan

74. Chronic Treatment with Azidothymidine (AZT) Alters Cytoskeletal Proteins Responsible for Cardiac Function.
Vasudeva Kamath¹, Kevin McAbee², Deborah L. Donahue³, John Tan⁴, Erliang Zeng⁵, Edward E. McKee¹,²,⁴
¹College of Medicine, Central Michigan University, Mount Pleasant, Michigan, United States, ²Indiana University School of Medicine, South Bend, Indiana, United States, ³W M Keck Center for Transgene Research, University of Notre Dame, Notre Dame, Indiana, United States, ⁴Department of Biological Sciences,
University of Notre Dame, Notre Dame, Indiana, United States, 3Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, Indiana, United States

75. Nucleozin Elicits Rapid and Target-Specific Aggregation of Influenza A Nucleoprotein (NP).
Richard Y Kao
The University of Hong Kong, Hong Kong, Hong Kong

76. Inhibition of Influenza Virus Entry by Epigallocatechin Gallate, the Green Tea Flavonoid.
Meehyein Kim1, So-Yeon Kim1, Hae Soo Kim1, Jin Soo Shin1, Woo Ghil Lee1, Pilho Kim2, Young-Sik Jung2, Chong-Kyo Lee1
1Virus Research and Testing Group, Korea Research Institute of Chemical Technology, Yuseong-gu, Daejeon, South Korea, 2Cancer and Infectious Diseases Therapeutics Research Group, Korea Research Institute of Chemical Technology, Yuseong-gu, Daejeon, South Korea

77. Identification of New And Novel Types of Inhibitors Against HIV-1.
Seon Hee Kim1, Sunhee Lee1, Hong Kim2, Jung Ae Park2, Ji Chang You1
1The Catholic University of Korea, Seoul, South Korea, 2Avixgen Inc., Seoul, South Korea

78. IC50s of Neuraminidase Inhibitors to Influenza Viruses are Highly Variable Dependent on Experimental Conditions.
Shuku Kubo, Satoko Tobiume, Makoto Yamashita
Daiichi Sankyo Co. Ltd., Shinagawa, Tokyo, Japan

79. A Novel Strategy for Efficient Production of Anti-V3 Human SCFVs Against HIV-1 Clade C.
Rajesh Kumar1, Raiees Andrabi1, Ashutoush Tiwari1, Prakash S S1, Naveet Wig,2, Anurag Sankhyan1, Subrata Sinha3, Kalpana Luthra1
1Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, Delhi, India, 2Department of Medicine, All India Institute of Medical Sciences, New Delhi, Delhi, India, 3National Brain Research Centre, Manesar, Harayana, India

80. Roles of NS5B Amino Acids 15, 223, and 321 in HCV Replicon Replication Using Mutagenesis and Crystallization of the NS5B Polymerase.
AM Lam1, S Bansal1, RT Mosley1, TE Edwards2, E Murakami1, MJ Sofia1, MJ Otto1, PA Furman1
1Pharmasset Inc., Princeton, NJ, United States, 2Emerald BioStructures, Bainbridge Island, WA, United States

81. Sublingual Administration of Lactobacillus rhamnosus Facilitates Protection Against Influenza Virus Infection in Mice.
Yu-Na Lee1, Ha-Na Youn1,2, Hyo-Sun Ju1,2, Ki-Taek Kim3, Joong-Bok Lee1, Seung-Yong Park3, In-Soo Choi1, Chang-Seon Song1
1Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, Korea, 2M21 Environmental Technology, Inc., Hwaseong, Gyeonggi-do, Korea, 3Gueulri Advanced Biotechnology, Ansan, Gyeonggi-do, Korea

82. Structural Insights into RNA 2'-O Methylation by the Flavivirus NS5 Protein.
Julien Lescar1,2, Lijian Yap1, Kayan Chung1, Pei-Yong Shi2, Siew Pheng Lim2
83. "Resistance Analysis and Characterization of a Thiazole Analogue, BP008, as a Potent Hepatitis C Virus NS5A Inhibitor".

Hui-Mei Lin, Jing-Chyi Wang, Han-Su Hu, Pei-Shan Wu, Chi-Chen Yang, Andrew Yueh
National Health Research Institutes, Zhunan, Miaoli, Taiwan

84. Structure-Based Inhibition of Norovirus RNA-Dependent RNA-Polymerases.

Eloise Mastrangelo1,2, Margherita Pezzullo3, Delia Tarantino1, Roberto Petazzi1, Romina Croci1, Jacques Rohayem3,4, Martino Bolognesi1, Mario Milani1,2
1University of Milano, Milano, Italy, 2CNR, Milano, Italy, 3Dresden University, Dresden, Germany, 4Riboxx GmbH, Radebeul, Germany

85. Mechanism of Ivermectin-Mediated Flaviviral Helicase Inhibition.

Eloise Mastrangelo1,2, Margherita Pezzullo3, Tine De Burghgraeve3, Johan Neyts4, Martino Bolognesi1, Mario Milani1,2
1University of Milano, Milano, Mi, Italy, 2CNR-IBF, Milano, Mi, Italy, 3University of Leuven, Leuven, Belgium, 4Belgium

86. Chemokine Receptors CXCR4 and CCR5 In HIV/HCV Coinfected Patients.

Natalia Matsiyeuskaya
Medical university, Grodno, Belarus


Eleftherios Michailidis1, Jordan Wilkins1, Emily M. Ryan1, Atsuko Hachiya2, Eiichi N. Kodama3, Hiroaki Mitsuya4,5, Michael A. Parniak6, Stefan G. Sarafianos1
1University of Missouri, Columbia, Missouri, United States, 2National Center for Global Health and Medicine, Tokyo, Japan, 3Tohoku University, Sendai, Japan, 4Kumamoto University, Kumamoto, Japan, 5National Institutes of Health, Bethesda, Maryland, United States, 6University of Pittsburgh, Pittsburgh, Pennsylvania, United States

88. Sustained Activity of 4'-Ethynyl Nucleosides to Variants with M184V Mutation in HIV-1 Reverse Transcriptase.

Fusako Miyamoto1, Kumi Kawaji1, Toshio Hatton1, Hiroaki Mitsuya2, Eiichi N. Kodama3, Eiichi N. Kodama1
1Tohoku University School of Medicine, Sendai, Miyagi, Japan, 2Kumamoto University, Kumamoto, Japan, 3University of Missouri, Columbia, Missouri, United States

89. GRL-007: a Novel Small Molecule CCR5 Antagonist Potent Against a Wide Spectrum of HIV-1.

Hiroto Nakata1, Debananda Das2, Kenji Maeda2, Kalapala V. Rao3, Arun K. Ghosh4, Hiroaki Mitsuya1,2
1Kumamoto Univ, Kumamoto, Kumamoto, Japan, 2ERS, NCI, NIH, Bethesda, Maryland, United States, 3Purdue Univ, West Lafayette, Indiana, United States

90. Ligand-Bound Structures of the Dengue Virus Protease Show the Active Conformation.

Christian G. Noble
Mika Okamoto¹, Xu Zhang², Takayuki Hamazaki¹, Masaaki Toyama¹, Yoshitaka Furukawa², Teruto Hashiguchi¹, Yasuo Suda³, Masanori Baba¹ 
¹Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Kagoshima, Japan, ²Kagoshima University Hospital, Kagoshima, Kagoshima, Japan, ³Graduate School of Science and Engineering, Kagoshima University, Kagoshima, Kagoshima, Japan

92. Establishment of In Vitro Culture System for Hepatitis E Virus (Genotype 3) Originating from Human Blood Plasma and Its Characterization. 
Takashi Owada¹, Moe Kaneko¹, Chieko Matsumoto¹, Kazuhiro Mio², Keiji Matsubayashi³, Shigeharu Uchida¹, Masahiro Satake¹, Kenji Tadokoro¹ 
¹Japanese Red Cross Society Central Blood Institute, Koto-ku, Tokyo, Japan, ²National Institute of Advanced Industrial Science and Technology, Koto-ku, Tokyo, Japan, ³Japanese Red Cross Society, Hokkaido Red Cross Blood Center, Sapporo, Hokkaido, Japan

93. Combination of Triterpenoids from *Platycodon grandiflorum* with Interferon-α Enhanced Suppression of Hepatitis C Virus Replication In Vitro and In Vivo. 
Sang Jin Park¹, Jae Won Yang¹, Joo Won Suh², Sang Wook Lee¹, Jong Woo Kim¹,² 
¹B&C Biopharm Co., Ltd., Suwon-si, Gyonggi-do, South Korea, ²Myongji University, Yongin-si, Gyonggi-do, South Korea

Todd B Parsley, Lu Yang, Robert W Buckheit, Jr. 
ImQuest BioSciences, Inc., Frederick, Maryland, United States

95. Preclinical Characterization of Miravirsen (MIR), a Novel Anti-HCV Therapeutic Targeting the Host Cell Factor miR-122. 
Amy K Patick¹, Todd B Parsley², Lu Yang³, Karin Zeh¹, Anneke K Raney¹, Michael R Hodges¹, Søren Ottosen² 
¹Santaris Pharma A/S, San Diego, CA, United States, ²Santaris Pharma A/S, Hørsholm, Denmark, ³ImQuest BioSciences, Frederick, MD, United States

96. Antiretroviral Agents Effectively Block HIV Replication After Cell to Cell Transfer. 
Marc Permanyer, Ester Ballana, Alba Ruiz, Bonaventura Clotet, Jose A. Este IrsiCaixa, Badalona, Barcelona, Spain

97. The Broad Spectrum Antiviral Activity of T-705 Is Extended to Norovirus. 
Joana Rocha-Pereira¹,², Dirk Jochmans³, Kai Dallmeier³, Pieter Leyssen³, Johan Neyts⁴, Maria S-J Nascimento¹,² 
¹Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal, ²Centro de Química Medicinal da Universidade do Porto, Porto, Portugal, ³Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

98. Design, Synthesis and Assay of Novel Mercaptobenzimidazole Derivatives Against the West Nile Protease Target.
99. Studies Of Dengue NS3 Protease Inhibitory Activity of Novel Isatin Derivatives. Periyasamy Selvam\textsuperscript{1}, Priya Srinivasan\textsuperscript{2}, Tanvi Khot\textsuperscript{2}, R. Padmanaban\textsuperscript{2}, M. Chandramohan\textsuperscript{3}
\textsuperscript{1}Devaki Amma Memorial College of Pharmacy, Malapuram-673634, Kerala, India, \textsuperscript{2}Microbiology and Immunology, Georgetown School of Medicine, Washington, Washington DC, United States, \textsuperscript{3}Kamaraj Liver Hospital and Research Centre, Madurai, Tamilnadu, India

100. Studies On HIV Integrase and HIV Integrase/LEDGF Inhibitory Activity of Ethanolic Fractions (F1-F6) of \textit{Morinda citrifolia} L Noni. Periyasamy Selvam\textsuperscript{1}, T Paul Pandi\textsuperscript{1}, Nouri Neamati\textsuperscript{2}
\textsuperscript{1}Devaki Amma Memorial College of Pharmacy, Chelembra, Malapuram DT, Kerala, India, \textsuperscript{2}Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angeles, CA 90089, United States

101. Inactivation of Arenavirus Infection by Aromatic Disulfides. Claudia S. Sepúlveda\textsuperscript{1}, Cybele C. García\textsuperscript{1}, Jesica M. Levingston Macleod\textsuperscript{2}, Nora López\textsuperscript{3}, Elsa B. Damonte\textsuperscript{4}
\textsuperscript{1}Laboratorio de Virología, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina, \textsuperscript{2}Centro de Virología Animal, I.C.T. Dr. César Milstein, CONICET, Buenos Aires, Argentina

102. Mammalian CellsPersistently Infected with Japanese Encephalitis Virus Return to Normal Phenotype on Curing with siRNA. Paresh S Shah, Cecilia Dayaraj, Atanu Basu, Deepak A Gadkari National Institute of Virology, Pune, Maharashtra, India

103. Influenza Virus Infections in Mice are Exacerbated by Intranasal Drug Delivery and are Difficult to Treat with Zanamivir. Donald F. Smee, Brett L. Hurst, Min-Hui Wong, E. Bart Tarbet Utah State University, Logan, Utah, United States

104. Evaluation of Influenza Virus Endonuclease Inhibitors by Cell Culture and Enzymatic Methods, Including a Novel Real-Time Fluorescence Assay. A. Stevaert\textsuperscript{1}, G. Rispoli\textsuperscript{2}, N. Pala\textsuperscript{3}, S.A.E. Marras\textsuperscript{4}, M. Sechi\textsuperscript{3}, L. Naesens\textsuperscript{1}
\textsuperscript{1}Rega Institute, KU Leuven, Leuven, Belgium, \textsuperscript{2}Dipartimento di Chimica Gen. ed Inorganica, Università di Parma, Parma, Italy, \textsuperscript{3}Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy, \textsuperscript{4}Public Health Research Institute, University of Medicine and Dentistry of New Jersey, Newark, NJ, United States

105. Heat Shock Protein 70 Inhibits HIV-1 Vif-Mediated Ubiquitination and Degradation of APOBEC3G. Ryuichi Sugiyama\textsuperscript{1}, Hironori Nishitsuji\textsuperscript{1}, Makoto Katahira\textsuperscript{2}, Hiroaki Takeuchi\textsuperscript{3}, Yuichiro Habu\textsuperscript{4}, Akihide Ryō\textsuperscript{5}, Hiroshi Takaku\textsuperscript{1}
\textsuperscript{1}Chiba Institute of Technology, Narashino, Chiba, Japan, \textsuperscript{2}Kyoto University, Uji, Kyoto, Japan, \textsuperscript{3}Tokyo Medical and Dental University, Bunkyou-ku, Tokyo, Japan, \textsuperscript{4}Colorado State University, Fort Collins, CO, United States, \textsuperscript{5}Yokohama City University School of Medicine, Kanazawa-ku, Yokohama, Japan
106. Cepharanthine and a Tetramethylnaphthalene Derivative Synergistically Inhibit HTLV-1-Infected Cell Proliferation In Vitro.
Masaaki Toyama\textsuperscript{1}, Takayuki Hamasaki\textsuperscript{1}, Tomofumi Uto\textsuperscript{1}, Hiroshi Aoyama\textsuperscript{2}, Mika Okamoto\textsuperscript{1}, Yuichi Hashimoto\textsuperscript{2}, Masanori Baba\textsuperscript{1}
\textsuperscript{1}Kagoshima University, Kagoshima, Kagoshima, Japan, \textsuperscript{2}The University of Tokyo, Bunkyo-ku, Tokyo, Japan

107. Molecular Modeling Studies on DENV Helicase.
Iuni M. L. Trist\textsuperscript{1}, Suzanne Kaptein\textsuperscript{2}, Pieter Leyssen\textsuperscript{2}, Johan Neyts\textsuperscript{2}, Andrea Brancale\textsuperscript{1}
\textsuperscript{1}Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, United Kingdom, \textsuperscript{2}Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium

108. HCV RdRp-Complex Simulation for the Understanding of Recognition Element Mediated by Pseudonucleoside Inhibitor.
Balaraju Tuniki, Chandralata Bal, Ashoke Sharon
Department of Applied Chemistry, Birla Institute of Technology, Ranchi, Jharkhand, India

Thomas Vercruyssse\textsuperscript{1}, Eline Boons\textsuperscript{1}, Tom Venken\textsuperscript{2}, Els Vanstreels\textsuperscript{1}, Arnout Voet\textsuperscript{2}, Marc De Maeyer\textsuperscript{2}, Dirk Daelemans\textsuperscript{1}
\textsuperscript{1}Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, \textsuperscript{2}Department of Biochemistry, Molecular and Structural Biology, KU Leuven, Leuven, Belgium

110. Dengue Virus Infection of Human Dermal Microvascular Endothelial Cells Is Inhibited by Sulfated \textit{Escherichia coli} K5 Polysaccharide Derivatives.
Peter Vervaekte\textsuperscript{1}, Marijke Alen\textsuperscript{1}, Dominique Schols\textsuperscript{1}, Pasqua Oreste\textsuperscript{2}, Sandra Liekens\textsuperscript{1}
\textsuperscript{1}Rega Institute, KU Leuven, Leuven, Belgium, \textsuperscript{2}Glycores 2000, Srl, Milan, Italy

Shaohui Wu, Chunming Lu, Fengxia Jiang, Shuang Er, Ning Ma, Xiaoquin Gai Liaoning CDC, Shenyang, Liaoning, China, China, China, China, China, China

112. Impact of Viral Sequences Beyond HCV NS5A Domain I on Potency of HCV NS5A Inhibitors.
Guangwei Yang, Yongsen Zhao, Dharaben Patel, Joanne Fabrycki, Milind Deshpande, Mingjun Huang
Achillion Pharmaceuticals, Inc, New Haven, CT, United States

113. Effets of the Combination of \textit{Lactobacillus rhamnosus} and Amantadine on Influenza A Virus Infection in Mice.
Ha-Na Youn\textsuperscript{1,3}, Yu-Na Lee\textsuperscript{1}, Hyo-Sun Ju\textsuperscript{1,3}, Ki-Taek Kim\textsuperscript{2}, Joong-Bok Lee\textsuperscript{1}, Seung-Yong Park\textsuperscript{1}, In-Soo Choi\textsuperscript{1}, Chang-Seon Song\textsuperscript{1}
\textsuperscript{1}Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, Korea, \textsuperscript{2}M21 Environmental Technology, Hwaseong, Gyeonggi-do, Korea, \textsuperscript{3}Gueulri Advanced Biotechnology, Ansan, Gyeonggi-do, Korea

114. Evaluation of the Effects of Bioflavonoids on Dengue Virus Type-2 Replication.
Keivan Zandi\textsuperscript{1}, Boon-Teong Teoh\textsuperscript{1}, Sing-Sin Sam\textsuperscript{1}, Pooi-Fong Wong\textsuperscript{2}, Mohd Rais Mustafa\textsuperscript{2}, Sazaly Abubakar\textsuperscript{1}
115. Effects of Fisetin and Naringenin Against Dengue Virus In Vitro Replication.
Keivan Zandi¹, Boon-Teong Teoh², Sing-Sin Sam¹, Pooh-Fong Wong¹, Mohd Rais Mustafa¹, Sazaly Abubakar¹
¹Tropical Infectious Disease Research and Education Center (TIDREC), Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur, KL, Malaysia, ²Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, KL, Malaysia

116. Discovery of Imidazopyridinylthioacetanilide Derivatives as Potent HIV-1 Inhibitors by Scaffold Hopping.
Peng Zhan¹, Xiao Li¹, Christophe Pannecouque², Jan Balzarini², Erik De Clercq², Xinyong Liu¹
¹Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Ji'nan, Shandong, China, ²Rega Institute for Medical Research, K.U.Leuven, Minderbroedersstraat, Leuven, Belgium

Wednesday, April 18, 2012

Poster Session 2: Herpes Viruses, Pox Viruses, Other Antiviral Agents and Medicinal Chemistry
Royton Hall CD, 3rd Floor

04:15 PM - 06:15 PM

117. NAOMI: a Molecular Modelling Tool for the Prediction of Nucleoside Analogues Activation.
Daniele Avancini, Andrea Brancale
Cardiff University, Cardiff, United Kingdom

118. Prodrugs of Neuraminidase Inhibitors with High Oral Bioavailability.
Gordon Amidon¹², Deepak Gupta¹, Sheeba/V Gupta², Crystal Jurkiewicz¹, Mindy Collins¹, John Hilfinger¹
¹TSRL, Inc, Ann Arbor, MI, United States, ²University of Michigan, Ann Arbor, MI, United States

119. Elucidation of the Anti-Herpetic Activity of Tenofovir, an Anti-HIV Selective Drug.
J. Balzarini¹, G. Andrei¹, C. Vanpouille², E. Balestra³, T. Cihlar⁴, C.-F. Perno³, R. Snoeck¹, L. Margolis⁵
¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²National Institutes of Health, Bethesda, MD, United States, ³Department of Experimental Medicine and Biochemical Science, University of Roma Tor Vergata, Rome, Italy, ⁴Gilead Sciences, Inc., Foster City, CA, United States

120. The Utility of Plethysmography for Measuring Lung Function in Mice Infected with HPAIV for Use in Antiviral Or Vaccine Studies.
Dale L. Barnard, Donald F. Smee, John D. Morrey
Utah State University, Logan, UT, United States

Marcella Bassetto¹, Valerio Gatti², Tine De Burghgraeve³, Pieter Leyssen³,
Johan Neyts\textsuperscript{3}, Romano Silvestri\textsuperscript{2}, Andrea Brancale\textsuperscript{1}
\textsuperscript{1}Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff, United Kingdom, \textsuperscript{2}University of Rome "La Sapienza", Rome, Italy, \textsuperscript{3}Rega Institute for Medical Research, Leuven, Belgium

122. Molecular Modelling Studies on the Vpg-Polymerase Complex of Enteroviruses.  
Michela Cancellieri, Andrea Brancale  
University of Cardiff, Cardiff, Wales, United Kingdom

123. Design, Synthesis and Biological Evaluation of Piperidine Substituted Triazine Derivatives as Potent HIV-1 NNRTIs. \textquoteleft\textquoteleft  
Xuwang Chen\textsuperscript{1}, Peng Zhan\textsuperscript{1}, Christophe Pannecouque\textsuperscript{2}, Erik De Clercq\textsuperscript{2}, Xinyong Liu  
\textsuperscript{1}Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road,250012, Ji	extquotesinglenan, Shandong, P.R., China, \textsuperscript{2}Rega Institute for Medical Research, K.U. Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

124. Cyclic Forms of Selected Acyclic Nucleoside Phosphonates are Particularly Less Active Than Their Parent Counterparts Against Epstein-Barr Virus Replication in P3Hr-1 Cells, But Not in Akata Cells. \textquoteleft\textquoteleft  
Natacha Coen, Sophie Duraffour, Robert Snoeck, Graciela Andrei  
Rega Institute for Medical Research, Leuven, Leuven, Belgium

125. Varicella-Zoster Virus Resistance to L-BHDU, a Dioxolane L-Nucleoside, Is Dependent on Thymidine Kinase. \textquoteleft\textquoteleft  
Chandrav De\textsuperscript{1}, Uma S. Singh\textsuperscript{2}, Chung K. Chu\textsuperscript{2}, Jennifer F. Moffat\textsuperscript{1}  
\textsuperscript{1}SUNY Upstate Medical University, Syracuse, NY, United States, \textsuperscript{2}University of Georgia, Athens, GA, United States

126. The Picornavirus Inhibitor Enviroxime Inhibits HCV RNA Replication \textit{In Vitro} by Inhibiting PI4KIII\ldots \textquoteleft\textquoteleft  
Leen Delang, Lotte Coelmont, Pieter Leyssen, Johan Neyts  
Rega Institute for Medical Research, KULeuven, Leuven, Belgium

127. Targeting HIV gp120: Design, Synthesis and Biological Evaluation of Novel Polyboronate Carbohydrate Binding Agents. \textquoteleft\textquoteleft  
Marco Derudas\textsuperscript{1}, Paul C. Trippier\textsuperscript{1}, Jan Balzarini\textsuperscript{2}, Christopher McGuigan\textsuperscript{1}  
\textsuperscript{1}School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, \textsuperscript{2}Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium

128. CYSTUS052, a Polyphenol Rich Plant Extract, Exerts Potent Anti-Influenza Activity by Preventing Viral Attachment to Host Cells in a Non-Pharmacological Manner. \textquoteleft\textquoteleft  
Christina Ehrhardt\textsuperscript{1}, Eike R. Hrincius\textsuperscript{1}, Karolin Droebner\textsuperscript{2}, Oliver Planz\textsuperscript{2}, Stephan Ludwig\textsuperscript{1}  
\textsuperscript{1}University of Muenster, Institute of Molecular Virology, Muenster, Germany, \textsuperscript{2}University of Tuebingen, Interfakultäres Institut für Zellbiologie, Tuebingen, Germany

129. Chemical Genomics Profiling of Host Kinase Inhibitors as Broad Spectrum Antivirals. \textquoteleft\textquoteleft  
Carrie Evans\textsuperscript{1}, Colm Atkins\textsuperscript{1,2}, James Noah\textsuperscript{1,2}
130. Synthesis of 4'-Ethynyl-2'-Deoxy-4'-Thioribonucleosides and Discovery of a Highly Potent and Less Toxic NRTI.
Kazuhiro Haraguchi1, Hisashi Shimada1, Keigo Kimura1, Hiromichi Tanaka1, Takayuki Hamasaki2, Masanori Baba2, Yung-Chi Cheng3, Jan Balzarini4
1Showa University, Tokyo, Japan, 2Kagoshima University, Kagoshima, Japan, 3Yale University, New Haven, Connecticut, United States, 4Katholieke Universiteit Leuven, Leuven, Belgium

Yohei Isono1, Norikazu Sakakibara1, Paula Ordenez2, Takayuki Hamasaki2, Masanori Baba2, Masahiro Ikejiri3, Tokumi Maruyama1
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132. Neonatal Herpes Caused by an Acyclovir-Resistant Herpes Simplex Virus Type 1.
Satsuki Kakiuchi1,3, Hajime Wakamatsu2, Kazuhiro Kogawa2, Shigeaki Nonoyama2, Naoki Inoue1, Masashi Mizuguchi3, Lixing Wang3, Masayuki Saigo1
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133. Creation of Universal Vectors for Prophylactic And/Or Therapeutic Recombinant Virus Vaccines.
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135. Crystal Structures of Murine Norovirus-1 RNA-Dependent RNA Polymerase in Complex with 2-Thiouridine Or Ribavirin.
Kyung Hyun Kim1, Intekhab Alam1, Ji-Hye Lee1, Mi Sook Chung2
1Korea University, Seoul, Korea, 2Duksung Women's University, Seoul, Korea

136. The Activity of New CAGE Compounds Against Influenza Viruses.
1Samara State Technical University, Samara, Samara rgn., Russia, 2The Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, Minsk rgn., Belarus

137. The Activity of the New Adamantane Derivatives Against the Orthopoxviruses.
Yuri Klimochkin3, Marina Leonova3, Vitaly Osyanin3, Eugene Golovin3, Eugene Belanov2, Sergey Balakhnin2, Olga Serova2, Nikolay Bormotov2
1Samara State Technical University, Samara, Samara rgn., Russia, 2FSRI SRC
138. Antiviral Activity of Oversulfated Smaller Molecules of Sulfated Exopolysaccharide from the Marine Microalga Gyrodinium impudicum Strain Kg03.

Chong-Kyo Lee¹, Meehyein Kim¹, Woo Ghil Lee¹, Jin Soo Shin¹, Hae Soo Kim¹, Jae-Seon Hwang¹, Joung Han Yim²

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139. Antiviral Action of Callus Extracts and Proteolytic Inhibitors of Plant Origin.

V. Lozitsky¹, A. Fedchuk¹, T. Grydina¹, L. Mudrik¹, L. Socheslo¹, N. Kuchuk², V. Belokurova², T. Torok³

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140. Action Mechanisms of Tricin on Anti-Cytomegalovirus Effect.

Tsugiya Murayama¹, Yuuzo Tuchida², Rie Yamada¹, Hidetaka Sadanari¹, Keiko Matsubara¹

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141. Antiviral Effect of the Specific Immunoglobulin Against HSV-1.

Nadiya V. Nesterova¹, Oksana V. Kurkina², Oksana V. Kurkina², Olga Yu. Povnitsa¹, Svitlana D. Zagorodnya¹, Galina V. Baranova¹

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142. Dippro-Nucleoside Diphosphate Prodrugs of 2',3'-Dideoxyuridine (DDU) and 2',3'-Dideoxy-2',3'-Didehydrouridine (D4U).

Florian Pertenbreiter¹, Jan Balzarini², Chris Meier¹

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143. Broad Spectrum Antiviral Activity of First Generation Methylene cyclopropane Analogs.

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144. Stereoselective Synthesis, Antiviral Activity and Stability of Methyl-Substituted Cyclosal-Pronucleotides.

Edwuin Hander Rios Morales¹, Jan Balzarini², Chris Meier¹

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Emily Scarbrough¹, Kurt Vermeire², Dominque Schols², Thomas Bell¹

¹University of Nevada, Reno, NV, United States, ²Katholieke Universiteit Leuven, Leuven, Belgium
146. Flavonoid Compounds Possessing Broad Spectrum of Antiviral Activities.  
DB Starosyla¹, Li Palchikovskaia², MP Zavelich³, AA Philchenkov³, LD Varbanets⁴, LD Zharkova¹, ST Diadiun¹, SL Rybalko¹  
¹LV Gromashevsky Institute of Epidemiology and Infectious Diseases of AMS of Ukraine, Kyiv, Ukraine, ²Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine, ³RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NAS of Ukraine, Kyiv, Ukraine, ⁴DK Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kyiv, Ukraine,

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149. Inhibition of Hepatitis C Virus Replication and Viral Helicase by Ethyl Acetate Extract of the Marine Feather Star Alloeocomatella polycladia.  
Atsuya Yamashita¹, Nobuyoshi Akimitsu², Naohiro Noda³, Satoshi Tsuneda⁴, Masayoshi Tsubuki⁵, Nobuyuki Enomoto⁴, Junichi Tanaka⁵, Kohji Moriishi⁶  
¹University of Yamanashi, Chuo, Yamanashi, Japan, ²The University of Tokyo, Bunkyo, Tokyo, Japan, ³National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan, ⁴Waseda University, Shinjuku, Tokyo, Japan, ⁵Hoshi University, Shinagawa, Tokyo, Japan, ⁶University of the Ryukyus, Nishihara, Okinawa, Japan, ⁷Japan, ⁸Japan

150. Comparative Analysis of Cytotoxicity of Fluorine-Containing Heterocyclic Compounds in Lymphoblastoid and Monolayer Cell Cultures.  
S. D. Zagorodnya¹, Yu. G. Shermolovich², N. V. Nesterova¹, A. V. Golovan¹, G. P. Gudz², L.O. Bilyavska¹  
¹Institute of Microbiology and Virology, NASU, Kyiv, Ukrenia, ²Institute of Organic Chemistry, NASU, Kyiv, Ukrenia

151. Efficacy of Tranylcypromine in Murine Models of Human Herpes Simplex Virus.  
Debra C. Quenelle¹, Mark N. Prichard¹, Jodi L. Vogel², Caroll Hartline¹, Deborah J. Collins¹, Terri L. Rice¹, Thomas N. Kristie²  
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Abstracts
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Oral Session 2: Hepatitis Viruses
Chairs: Timothy Block, Ph.D. and Takaji Wakita, Ph.D.
2:15 - 4:15 pm
Royton Hall AB, 3rd Floor

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FMCA Phosphoramidate Prodrug: In Vitro Antiviral Activity Against Lamivudine, Adefovir and Entecavir Resistant HBV Mutants and Preliminary In Vivo Anti-HBV Activity Against the Entecavir-Lamivudine Resistant Triple Mutant in Chimeric Mice

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Long-term anti-HBV nucleos(t)ide therapy is often associated with drug resistance which significantly compromises the clinical application of these agents. Therefore, novel antiviral agents active against drug-resistant HBV are critically needed. In order to search for nucleosides active against drug-resistant HBV, modifications with fluorine substitution on the 2'-position of carbocyclic nucleosides were carried out. FMCA and its phosphoramidate were synthesized according to the newly developed synthetic procedures. The synthesized compounds were evaluated against wild type HBV as well as lamivudine-, adefovir- and entecavir-resistant HBV mutants. FMCA and its prodrug maintained antiviral potency against adefovir- and lamivudine-resistant HBV mutants (N236T and M204V, M204I, L180M, M204I/I/V, respectively) in vitro. Furthermore, FMCA & its prodrug were also active against the entecavir/lamivudine triple mutant (L180M/M204V/S202G). The potency of the monophosphate prodrug was 10 fold greater than that of the nucleoside against wild-type (WT) as well as drug-resistant mutants. IHidden formatting deleted. Delete this text! bold"n order to determine the anti-HBV activity in vivo, FMCA phosphoramidate was evaluated in chimeric mice infected with WT and the entecavir/lamivudine triple mutant. The prodrug reduced the serum HBV DNA level by 2.2 log copies in mice infected with the WT clone, while in mice infected with entecavir/lamivudine-resistant clone, reduction of 1.2 log copies was observed. In view of the observed significant anti-HBV activity in vitro and in vivo against wild-type as well as the drug-resistant mutants without significantly increase of mitochondrial toxicity, their preclinical studies are warranted (Supported by NIH grant AI-25899, NOI-AI30046 and HIV/AIDS H22-AIDS-004).

13
TARGETED DELIVERY OF INTERFERON ALPHA TO HBV-INFECTED HEPATOCELLS BY USING T CELL RECEPTOR-LIKE MONOCLONAL ANTIBODIES

Changhua Ji, Sastry K. Seetharaama, Georg Tiefenthaler, Han Ma, Stefan Ries, Klaus Klumpp, Erhard Kopetzki
HBV is susceptible to the antiviral effect of type I and type II interferons but the effectiveness of these cytokines during chronic HBV infection is reduced, as chronic HBV is associated with suppressed anti-viral innate and adaptive immune responses. To explore if immune defects could be circumvented and interferon efficacy against HBV infection could be increased, we generated novel recombinant IFNα molecules that are specifically targeted to HBV-infected hepatocytes. These molecules consist of IFNα fused to monoclonal antibodies specifically recognizing peptide/MHC of HBV surface (S 183-91/A201) or core (core 18-27/A201) antigens presented on HBV infected cells. These antibodies mimic the MHC/peptide complex recognition by the T cell receptor (TCR) of HBV-specific CD8 T cells, thus called TCR-like antibodies (TCRL). These TCRL demonstrated specific binding to HBV-positive hepatoma cells and hepatocytes derived from HBV-infected patients. The TCRL-IFNα molecules selectively bound to HBV(+) cells in a co-culture containing HBV(+) and HBV(-) cells at 1:1 ratio. IFNα fusion did not alter the sensitivity of TCRL to cells expressing HBV antigens, while the intrinsic activity of the fused IFNα was reduced by 97%, resulting in selective induction of interferon responsive genes in HBV(+) cells, as compared to HBV(-) cells. The IFNα activity was markedly enhanced on hepatoma cells expressing HBV antigens but not on HBV antigen(-) cells. This enhanced IFNα activity was also observed on HBV-infected primary human hepatocytes. Pre-blocking of the MHC/peptide sites with TCRL abrogated the enhanced IFNα activity of TCRL-IFNα, indicating that TCRL binding to HBV peptide presenting MHC was required for enhanced IFNα activity. The low IFNα activity of TCRL-IFNα on non-target cells and its substantially higher IFNα activity than peg-IFNα on HBV-infected hepatocytes suggest these TCRL-IFNα molecules may be able to deliver high intrahepatic IFNα activity, with low systemic IFNα effects.

14
A Serum Factor is Critically Required for Efficient Synthesis of Hepadnaviral cccDNA

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HBV covalently closed circular DNA (cccDNA) functions as viral transcription template and its presence is required for maintaining chronic HBV infection. The ability to synthesize cccDNA and the synthesis efficiency in infected cells are controlled by the host. Little is known about how the synthesis of cccDNA is regulated by the host factors. The currently approved anti-HBV drugs such as nucleotide/side analogs inhibit HBV DNA replication at the RT step, but do not directly target HBV cccDNA, or impact the host's ability to support cccDNA synthesis. Thus, the current therapy does not actually clear chronic HBV infection. An agent that can be used for directly interfering with HBV cccDNA synthesis is highly sought. We have identified a serum factor that is critically required for efficient synthesis of cccDNA during hepadnaviral infection. DHBV infectivity is severely impaired if this specific serum component is degraded and viral infectivity is completely restored when uninfected serum is added to compensate for the degraded component. The loss of viral infectivity following degradation of the factor involves significant or complete loss of cccDNA synthesis without impact on the viral entry process. Furthermore, amplification of the cccDNA pool after established viral infection is also critically dependent on the level of this serum protein. Our evaluations utilizing a total of 70 duck serum samples with variable amounts of this serum protein have highlighted the strong correlation between levels of this serum factor and synthesized cccDNA in primary hepatocyte culture. Our data imply that quantitation of this protein could quantitatively reflect the host's ability to support cccDNA synthesis. This discovery represents a breakthrough in understanding how cccDNA synthesis is controlled by the host's factor and yields two immediate clinical applications: (1) Establishment of a bioassay to quantify the serum...
protein level in blood in order to monitor an individual patients' ability to support cccDNA synthesis and project cccDNA synthesis kinetics, onsets of acute exacerbation, and variant breakthroughs. (2) Provide a critical target for the development of a new generation of anti-HBV drugs that can directly interfere with cccDNA synthesis.

15
Human pluripotent stem cell derived hepatocyte progeny support complete replication of hepatitis C virus

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Current in vitro culture systems for HCV depend chiefly on human hepatoma cell lines. Although primary human hepatocytes support HCV infection in vitro the use of such models is limited because of shortage of human livers. Therefore, there is significant interest in the establishment of a HCV culture system in human stem cell-derived hepatocyte-like cells. We demonstrate that human embryonic stem cell (hESC)-derived hepatocytes can be infected with the HCV JFH1 genotype 2a, resulting in the production of viral RNA in the stem cell progeny. Inoculations were performed on day 23 progeny of hESC that were differentiated to hepatocytes. About 5% of hESC progeny were ALB+/AFP- cells, suggestive of a mature hepatocyte phenotype, whereas 30-40% of hESC-progeny stained positive for ALB as well as AFP (immature hepatoblasts). The percentage of ALB+ or CYP3A4/5 cells wherein NS5A could be detected was approximately 15%. Interestingly, in livers of patients (with a viral load > 5 log10 HCV) only a fraction (~7-20%) of hepatocytes is infected with HCV. Stem cell-derived hepatocytes produced for more than 10 consecutive days HCV core protein as well as virions that were capable of re-infecting hepatoma cells. Viral replication was selectively inhibited by a non-nucleoside HCV polymerase-inhibitor (HCV-796), a cyclophilin binding molecule (Debio 025, Alisporivir) and the protease inhibitor VX-950 (Telaprevir). This use of hESC-derived hepatocytes may be of great importance to study the biology of HCV replication (and that of other hepatotropic viruses) as well as the inhibition thereof by specific antivirals. A further optimization of the model will be needed to increase the efficiency of infection and to allow infection with clinical isolates of various genotype. This would allow to assess the in vitro efficacy of known and novel inhibitors of HCV on the replication of different genotypes.

16
Discovery of novel HCV inhibitors targeting the viral NS4B

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The current standard of care (SOC) for chronic HCV infection, pegylated interferon a and ribavirin, has limited efficacy and serious side effects. Recently, two HCV protease inhibitors, Victrelis and Incivek, have been approved for the treatment of HCV genotype 1 infection. In addition, a number of direct-acting antivirals have demonstrated encouraging efficacy in clinical studies. The discovery of new HCV treatments with novel mechanisms of action and enhanced combination activity remains essential to address the potential for resistance and limited efficacy observed in the clinic with current SOC. We have identified highly potent and selective small molecule inhibitors of HCV replication that act on the viral non-structural protein 4B (NS4B), an unexploited target. HCV NS4B is an integral membrane protein and plays essential roles in viral replication.
PTC-971 is a lead compound with an EC$_{50}$ of 6.6 nM and EC$_{90}$ of 33 nM against HCV genotype 1b replicon with a selectivity index of >500-fold with respect to cytotoxicity. De novo selection of HCV replicon resistance to this compound identified the substitutions F98C and V105M in NS4B that confer resistance to PTC-971 and its analogs. In enzymatic assays, PTC-971 is not active against HCV protease, helicase or polymerase. Consistent with these findings, PTC-971 does not select for cross resistance to inhibitors of HCV protease or polymerase. PTC-971 has enhanced activity against the HCV replicon in combination with interferon a, and inhibitors of HCV protease or polymerase. PTC-971 has good oral exposure in rats, dogs and monkeys with an oral bioavailability of 35 to 91%. PTC-971 is well distributed in the liver with a liver/plasma ratio of 11 to 1 in the rat. In a single-dose oral study in rats, the plasma exposure of PTC-971 increased with increasing doses. In a 14-day dosing study in rats, PTC-971 had a non-proportional increase of plasma exposure with no significant toxicity, clinical pathology or histopathology observed at doses of up to 2000 mg/kg, the highest dose evaluated. In summary, we have identified potent and selective HCV NS4B inhibitors with the potential to be used as part of a combination therapy with the SOC or other direct-acting antivirals to treat chronic HCV infection.

17
Discovery of a Novel Non-Nucleoside Inhibitor of HCV NS5B Which Possesses Broad Genotypic Potency and an Attractive Pre-Clinical Profile

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Non-nucleoside inhibitors (NNIs) of HCV NS5B bind sites distinct from the active-site pocket, making NNIs attractive as candidates for co-administration with nucleoside analogues or inhibitors of other targets in HCV replication in pursuit of an all-oral combination of Direct-Acting Antiviral Agents (DAA) to replace current Interferon –based regimens. Several NNIs are in clinical trials, typically demonstrating limited success due to both modest efficacy and restrictions in genotype coverage. In this work, a concerted medicinal chemistry effort sought to capture greater binding interactions within a central pocket on the NS5B surface between the 'palm' and 'thumb' domains, chemically extending a central benzofuran core previously shown to bind within this pocket. The strategy's success was confirmed using an early lead which through resistance selections simultaneously elicited mutations at both amino acids C316 and M414, previously shown to independently confer resistance to benzofuran- and thidiazine-based inhibitors. Further optimization of this series yielded Compound A, an NNI which demonstrated low nM potency across a broad panel of HCV genotypes and NNI resistance mutants. Compound A also possesses a good pharmacokinetic profile. More specifically, oral administration to rat or dog yielded AUCs (uM.hr) of 29.3 in rat (10 mg per kg) and 4.83 in dog (5 mg per kg). Importantly, the in vitro potency translated to in vivo efficacy. Oral dosing of Compound A to two HCV-infected chimpanzees, one each infected with gt1a or gt1b virus, at 2 mg per kg q.d. for seven days resulted in a rapid 2 log reduction in viral load with no evidence for viral resistance. Molecules in this series as exemplified by Compound A offer an attractive profile for clinical evaluation for combination with other DAA.

18
Metabolic Activation of the Anti-Hepatitis C Virus Nucleotide Prodrug, PSI-352938

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PSI-352938 is a novel cyclic phosphate nucleotide prodrug of b-D-2'-deoxy-2'-a-fluoro-2'-b-C-methylguanosine 5'-monophosphate with potent anti-HCV activity both in vitro and in vivo. In order to inhibit the HCV NS5B RNA-dependent RNA polymerase as a non-obligate chain terminator, PSI-352938 must be metabolized to the active triphosphate form, PSI-352666. Cell based metabolism studies demonstrated that a significantly higher amount of PSI-352666 was formed in primary hepatocytes than in Huh 7-derived Clone A HCV replicon cells. Cell based metabolism and biochemical assays were performed to identify the metabolic pathway of PSI-352938 in primary hepatocytes and the enzymes involved in the pathway. The first step, removal of the isopropyl group on the 3',5'-cyclic phosphate moiety was found to be cytochrome P450 (CYP) dependent. Among the CYP isoforms tested, CYP3A4 was the only enzyme that was capable of catalyzing the reaction. Metabolism of PSI-352938 was also inhibited by a selective CYP3A4 inhibitor, ketoconazole in primary human hepatocytes. After removal of the isopropyl group, the second step was hydrolysis of the cyclic phosphate by phosphodiesterases (PDEs). Among all the PDE families, PDE2A, PDE5A1, PDE9A, and PDE11A4 were tested as they are known to be expressed in liver and all of them were able to hydrolyze the cyclic phosphate. The O'-ethyl group in the guanine base is then hydrolytically removed by adenosine deaminase-like protein 1. The resulting monophosphate is consecutively phosphorylated to the diphosphate and to the active triphosphate metabolite, PSI-352666, by guanylate kinase 1 and nucleoside diphosphate kinase, respectively. In addition, formation of nucleoside metabolites was observed in primary hepatocytes and ecto-5'-nucleotidase was able to dephosphorylate the monophosphate metabolites. Since metabolism of PSI-352938 requires CYP3A4, which is highly expressed in primary hepatocytes, this supports the notion that PSI-352938 is activated in the liver.

19
Identification of a novel resistance mutation for hepatitis C virus benzimidazole inhibitor JT-16
Leen Delang, Mathy Froeyen, Johan Neyts
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The last decade great progress has been made in the development of direct-acting antivirals (DAA) against HCV. The RNA-dependent RNA polymerase of HCV is essential for viral RNA replication and is thus an excellent target for DAA. Non-nucleoside polymerase inhibitors based on a benzimidazole or indole scaffold have been reported. Compounds of this class can inhibit HCV replication by interacting with thumb domain 1 of the HCV polymerase. Escape mutants that confer resistance to these inhibitors in vitro map to amino acids P495, P496 or V499. We report a novel resistance mutation (T389S/A) that was identified following resistance selection with the benzimidazole non-nucleoside polymerase inhibitor JT-16 in a HCV genotype 1b subgenomic replicon. Clonal sequencing analysis of the JT-16™ replicon revealed that the T389S mutation was present in 60% of all clones sequenced, whereas the P495A mutation was only identified in 2 clones (= 13%). Introduction of mutations T389A or T389S into a wild-type backbone induced moderate levels of resistance to JT-16 (7- and 13-fold, respectively). In contrast, P495A is associated with high level resistance (44-fold). Furthermore, the replication fitness of the T389S mutant was significantly higher than that of P495A. By means of molecular modelling a structural hypothesis was formulated to explain the emergence of the T389S/A mutation in the JT-16 resistant replicon. A ligplot interaction map of residue T389 showed that T389 makes H-bonding interactions with C488, D387 and K491. Mutation of threonine 389 into an alanine or serine would disrupt this H-bonding network and possibly induce a change of rotameric state of the side chain of K491, thereby interfering with the binding of the JT-16 inhibitor. In conclusion, we demonstrated that less resistant, but fitter variants can develop during in vitro resistance selection with the benzimidazole inhibitor JT-16. Surprisingly, the previously published signature mutation for benzimidazole resistance, P495A, was only detected in 13% of the resistant population. Our data show that structural
modifications to inhibitors with a similar scaffold can affect the (pattern of) resistance mutations that emerge during resistance selection.

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HCV entry is a highly coordinated process involving viral and host cell factors, which is required for initiation, spread, and maintenance of viral infection. HCV entry is thus a promising target for anti-HCV therapy. The HCV envelope surface glycoproteins E1 and E2 are highly glycosylated and essential for HCV entry. Targeting the glycans on these envelope proteins may represent a useful therapeutic concept for controlling HCV infection. We have shown that the 25-kDa red algal lectin, griffithsin (GRFT) (a domain-swapped dimer of 12.7-kDa subunits) inhibits HCV infection at subnanomolar concentrations (~0.4 nM) in HCV cell culture assay with an impressive selectivity index of >90,000. GRFT inhibited HCV pseudoparticle (HCVpp) infections with various genotypes, while having no significant effect on viral replication in the HCV replicon assay. These results indicate that GRFT has its effect at the point of viral entry. As expected by its reported carbohydrate binding property, GRFT bound to the HCV envelope glycoprotein E2 in an ELISA-based assay confirming the molecular target for GRFT. GRFT was shown to be bioavailable following subcutaneous injection into mice with plasma GRFT concentrations reaching a level ~600-fold higher than the in vitro EC50. We demonstrate that GRFT shows in vivo efficacy in reducing HCV titers in a human hepatocyte-engrafted, Alb-uPA/SCID mouse system. Single agent treatment with GRFT once daily for 18 consecutive days resulted in a 4-log reduction in HCV viral titers. The anti-viral response was stable for 30 days and no viral rebound was observed. These results indicate that HCV entry inhibitors can be effective agents in anti-HCV therapy.

Oral Session 3: Respiratory and Emerging Infections
Chairs: Simon Tucker, Ph.D. and Johan Neyts, Ph.D.
8:45 - 11:45 am
Royton Hall AB, 3rd Floor

22
Novel Fusion Inhibitors of Influenza Virus

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Oseltamivir remains the most used neuraminidase inhibitor (NAI) for treatment of influenza virus infection. Meta-analysis of 15 studies yielded a pooled incidence rate for oseltamivir resistance of 2.6%. A new inhibitor of a different target will provide defense against emergence of potential NAI-resistant viruses. We focused on small molecule fusion inhibitors. We conducted extensive SAR studies and identified a few classes of fusion inhibitors with EC50 values less than 1.0 nM based on plaque assays. The fusion inhibitors are efficacious against all strains of
influenza virus tested, including H1N1, H3N2, H1N9, H5N3 (vaccine reassortant) and type B viruses. Selective index is higher than 100,000. The inhibitors bind influenza virus virions, but do not cause any damage in particle morphology. Mechanistic studies by hemolysis and live imaging showed that our novel inhibitors blocks virus entry at the membrane fusion step. A number of in vivo studies using a mouse model for influenza virus infection were performed to evaluate these inhibitors. Oseltamivir was used as a positive control. Our fusion inhibitors administered orally have been shown to increase the survival rate upto 80% after a lethal infection of the mice with A/PR/8 virus. These fusion inhibitors are as efficacious against wild type influenza viruses as against NAI-resistant or amantadine-resistant variants. Synergy in combination with oseltamivir was clearly demonstrated when the dose of oseltamivir was lowered below its effective level. Pharmacokinetics studies are employed to help optimization. Our studies suggest that fusion inhibitors may be a promising candidate for combination therapy together with NAI.

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DISCOVERY AND DEVELOPMENT OF ORALLY ACTIVE ANTIVIRALS FOR THE TREATMENT OF RSV: IDENTIFICATION OF A 2nd GENERATION CANDIDATE

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Biota Scientific Management, Pty, Ltd, Melbourne, Australia

Background: Respiratory syncytial virus (RSV) is the predominant cause of acute lower respiratory tract infection (LRTI) in children. It has been estimated that in the United States 4-5 million children up to four years of age will develop an acute RSV LRTI annually. More than 125,000 children are admitted to hospital for RSV related illness in the United States each year. RSV infection is also a major cause of morbidity and mortality in high risk adult populations where infection rates can range from 50-80% depending on the underlying medical condition. Based on a prospective surveillance study of hospitalised, elderly and high-risk adult patient groups, it is estimated that RSV infection accounts for approximately 177,525 hospital admissions annually in the United States. Biota has discovered and developed a suite of orally dosed, small molecule fusion inhibitors for the treatment and prevention of RSV infection. Biota’s RSV inhibitor program encompasses several classes of RSV fusion inhibitors and a 1st generation candidate, BTA9881, was advanced into Phase I clinical trials.

Methods and Results: Data will be presented describing the development of a new series of RSV inhibitors and the identification of a potent and selective 2nd generation preclinical candidate. This candidate demonstrates improved potency against RSV A&B strains (EC50 <100nM in cytopathic effect and plaque reduction assays for laboratory
strains and clinical isolates) and a resistance profile consistent with inhibition of the RSV fusion glycoprotein. The compound displays dose responsive reductions in RSV lung viral titers in the cotton rat and Balb/c mouse moreover, in the Balb/c mouse model, the candidate significantly reduced levels of RSV-mediated inflammatory markers including inflammatory cells, cytokines, chemokines and growth factors. The candidate has desirable cross-species ADME properties in vitro (e.g. high metabolic stability and low protein binding) and excellent pharmacokinetics following intravenous and oral administration (e.g. oral bioavailabilities of 72% and ~100% for rat and dog respectively).

**Conclusions:** A new small molecule drug has been discovered for treatment of RSV infections with potential to be dosed by the oral and intravenous routes. Biota’s 2nd generation candidate displays potent and selective inhibition of the fusion glycoprotein. The compound has excellent pharmacokinetic and safety profiles in multiple species, is effective in animal models of RSV disease and is now undergoing preclinical regulatory studies.


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**Immune Response to Fluzone® and FluMist® Vaccines in Balb/c Mice and Efficacy Against Influenza**

**A/CA/04/2009 (pandemic H1N1) Virus Challenge**

Bart Tarbet, Brett Hurst, Bentley Anderson, Tyler McLean, John Morrey

Utah State University, Logan, USA

These studies evaluated the immune response following immunization with the trivalent inactivated influenza vaccine (Fluzone®) and trivalent live attenuated vaccine (FluMist®) in BALB/c mice. Vaccine efficacy was also evaluated against challenge infection with influenza A/CA/04/2009 (pandemic H1N1) virus. Mice were vaccinated IM with Fluzone® or IN with FluMist® at three concentrations of each vaccine. These results will be used as a baseline of vaccine efficacy for future experimental vaccine studies. Both vaccines provided excellent protection against challenge infection. Evaluation of humoral immunity following vaccination included measurement of IgA levels in lung lavage, and serum antibody levels by hemagglutination inhibition (HAI) and total IgG assays. Cellular immunity was evaluated by quantitation of cytokines and chemokines in lung lavage fluids by multiplex ELISA. All groups receiving Fluzone®, and the FluMist® groups receiving the two highest vaccine concentrations showed significant increases in HAI titers over placebo on day 14 post-vaccination. Although, the HAI titer induced by vaccination with the highest dose of Fluzone® was significantly higher than the titer induced by the highest dose of FluMist®. In addition, the group vaccinated with the highest dose of Fluzone® showed a significant increase in IgA, and all groups receiving FluMist® were significantly higher than placebo on day 14. The groups vaccinated with the two highest concentrations of Fluzone® also showed a significant increase in IgG, and all groups receiving FluMist® were significantly higher than placebo on day 14. On day 3 post-challenge, all concentrations of both vaccines decreased levels of IL-2, IL-5, IL-6, IL-10, IL-17, IFN-γ, and MCP-1. On day 6 post-challenge, all concentrations of both vaccines decreased levels of IL-2, IL-6, IL-17, IFN-γ, and MCP-1. In addition, the two highest concentrations of both vaccines decreased GM-CSF on day 6. The only cytokine levels that showed significant increases were on day 6 for IL-4 and IL-5 in the Fluzone® vaccinated groups. [Supported by contract HHSN272201000039I from the Respiratory Diseases Branch, DMID, NIAID, NIH, DHHS]
Therapeutic Efficacy of ST-246 in Cynomolgus Macaques Challenged with Monkeypox Virus via Aerosol

Lovelace Respiratory Research Institute, Albuquerque, USA

Monkeypox virus (MPXV) is a biological agent that could be used as a bioweapon. It is also a useful surrogate for smallpox (VARV) in studies of potential medical countermeasures against this agent, which is regarded as one of the most devastating pathogens of human history. The deliberate release of VARV is regarded as a possible threat by the US government. VARV is highly contagious and can cause severe disease and death in humans. Human trials on candidate antiviral drugs cannot be done for either VARV or MPXV so well characterized animal models of severe orthopoxvirus disease are needed. Non-Human Primates (NHPs) were treated orally once daily for 14 days with 10mg/kg ST-246 with separate groups starting 1 to 8 days post MPXV aerosol challenge with 1x10⁷ PFU/NHP. Clinical observations were made twice daily for up to 45 days post challenge. Pharyngeal swab and blood analysis, clinical pathology, chest x-rays, and lesion counts were conducted at routine intervals. Tissues were collected from each animal at necropsy for determination of viral load and histopathological analysis. All NHPs for which treatment was initiated 1 to 5 or 7 days after exposure survived to the end of the study while 4 of 6 NHPs for whom treatment was initiated on day 6 and 3 of 6 animals for whom treatment was initiated on day 8 survived to the end of the study. Only 2 of 8 controls survived, with a mean time to death of 11 days, and a range of 8-12 days. Severity of illness in the treated NHPs correlated with the delay of treatment. Treated animals exhibited changes in hematology and clinical chemistry consistent with but less severe than those observed in control NHPs and also showed moderate weight loss and elevated respiration rates which correlated with delay of treatment. Delay of treatment also correlated with increased presence of virus in pharyngeal swabs. Results indicate that treatment with ST-246 delayed up to 8 days following MPXV challenge significantly improves survival over untreated control animals even late in infection and delay of treatment is correlated with an increase the severity and incidence of observed signs of disease.

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TP219 acts as a selective inhibitor of in vitro enterovirus replication by indirectly targeting morphogenesis

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¹Rega Institute for Medical Research, KU Leuven, Belgium, ²Radboud University Nijmegen Medical Center, Nijmegen, Netherlands, ³Vrije Universiteit Brussel, Brussel, Belgium, ⁴Instituto de Química Instituto de Química Médica, Madrid, Spain

We identified a class of 9-arylurines as selective inhibitors of the replication of CVB3 and several CVAs. Analogue TP219 [9-(3-acetylphenyl)-6-chloropurine], was selected for further studies. TP219 inhibits virus-induced CPE formation, without affecting (i) viral RNA synthesis, (ii) polyprotein synthesis/processing or (iii) the formation of replication vesicles. No infectious virus particles were detected in TP219-treated infected cells, suggesting that the drug interferes with the formation of novel infectious viral progeny. TP219-resistant (TP219¹⁰) CVB3 was selected and was shown to carry several mutations in VP1 (T77M, V150I and N212S) and VP3 (A180T). T77M proved sufficient for the drug-resistant phenotype. T77M (and the other mutations) is/are located at the interface of two
protomers, suggesting that the molecule may prevent (directly or indirectly) viral morphogenesis. By means of sucrose gradient ultracentrifugation and SDS-PAGE data were collected that suggest that TP219 prevents promoter-promoter interaction. Interestingly, the mutant virus proved cross-resistant with L-BSO, a known inhibitor of the glutathione (GSH) biosynthesis. Addition of GSH-reduced-ethyl-ester (GEE) to the culture supernatant reversed the antiviral effect of TP219. Next, we demonstrated that TP219 rapidly and efficiently reduces intracellular levels of GSH. This was corroborated by the observation that TP219 and GSH, when incubated in cell free conditions, form an adduct via a covalent bond. The effect of an oxidizing environment (as a result of the depletion of intracellular GSH) on VP1, and more specifically on T77, is currently being explored by means of MALDI-MS and nanoLC-MS. Oxidation of T77 may possibly be detrimental for protomer:protomer interaction. Taken together, TP219 provides a most exciting tool to obtain insights into a poorly understood part of enterovirus replication.

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A new mouse model of Chikungunya virus with utility in antiviral studies

Justin Julander1, Ashley Dagley2, Jane Ennis2, John Morrey1, Jeff Turner1

1Utah State University, Logan, USA; 2Defyrus Inc., Toronto, Canada

Chikungunya virus (CHIKV) is an alphavirus that causes a debilitating arthralgia in the majority of those infected, which can last for weeks to years after infection. There have been several large-scale outbreaks recently, causing significant morbidity and mortality. Mortality rates are generally low, but most infections are symptomatic. Since there is no treatment for this disease, it is important that an animal model is developed that is useful in the evaluation of potential antiviral compounds. Infection of 5 different mouse strains identified the DBA/1J strain as a suitable model for CHIKV studies. Unlike the other mouse strains tested, the DBA/1J strain is readily available and exhibits disease similar to that found in man. Mice infected via footpad/hock injection with CHIKV (10⁶ CCID₅₀ of strain S27) experience a swelling of the joints at the site of inoculation, which was associated with an increase in inflammatory cytokines with a peak at maximal swelling. The most reliable non-invasive parameter relevant to human disease was a measureable increase in footpad thickness that had a peak swelling 7 days after virus challenge. Virus titer is detectable in spleen, serum, and in the inoculated limb for 2-3 days after inoculation, but is cleared from the system thereafter. Treatment of mice 24 h prior to virus challenge with 10⁷ pfu of mDEF201, an adenovirus-vectorized interferon, resulted in a significant (P<0.001) decrease in footpad swelling. Virus titers of the hind limb, which was inoculated with virus, and in the spleen were also significantly reduced in mice treated with the 10⁷ pfu dose of mDEF201. Average spleen weights were also improved in a dose dependent fashion, although this improvement was not significant. Ribavirin treatment at a dose of 75 mg/kg/d had no effect on disease in CHIKV-infected mice. In summary, infection of DBA/1J mice with CHIKV appears to be a suitable model for the evaluation of antiviral agents in the treatment of relevant disease. In addition, prophylactic treatment with the adenovirus-vectorized interferon, mDEF201, resulted in significant improvement in disease parameters. [Supported by HHSN272201000039I Task Order A21 from the Virology Branch, NIAID, NIH]

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Inhibitor of Human Coronavirus 229E Infectivity Targeting Viral Non-structural Protein 6 Involved in Modulation of Cellular Membranes

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1Department of Clinical Virology, University of Gothenburg, Gothenburg, Sweden; 2Organic Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
Coronavirus, an enveloped, positive stranded RNA virus, have been reported to modify intracellular membranes to form the reticulovesicular network (RVN) for virus replication in cytoplasm and/or to provide binding sites for attachment of the viral replicase complex at the RVN. In the case of CoV 229E it is believed that the hexapasin non-structural protein 6 is, at least in part, involved in this cellular membrane modification. In this study we have identified a potent inhibitor of coronavirus 229E infectivity, referred to as K22, which displayed an antiviral IC50 value of 0.7 μM while showing no toxicity for MRC-5 cells at 20 μM (solubility limit). Results of the time-of-addition and RT-PCR assays suggested that the drug inhibited viral infection in MRC-5 cells at a stage of the infectious cell cycle occurring after virus entry into the cells, and manifested as near-complete inhibition of the viral RNA synthesis. When further evaluating the mechanism of action of this substance we observed that K22 appeared to inhibit formation of the virus-induced clusters of the double membrane vesicles of RVN. Furthermore, two independently generated viral variants resistant to K22 exhibited amino acid substitutions of H121L and M159V, respectively, both occurring in the viral non-structural protein 6 suggesting that this compound could interfere with the membrane modifying activity of this protein. Altogether, K22 is an anti-coronavirus 229E lead compound with a novel mechanism of action which, in addition to its antiviral activity, may help to clarify some features of coronavirus life cycle.

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The RAFIs aUY11 and dUY11 Inhibit Infectivity of Unrelated Enveloped Viruses by Preventing Fusion of Viral and Cellular Membranes

Che C. Colpitts, Alexey V. Ustinov, Vladimir A. Korshun, Luis M. Schang

1University of Alberta, Edmonton, Canada, 2Russian Academy of Sciences, Moscow, Russia

We have described a family of novel antiviral compounds, the rigid amphipathic fusion inhibitors (RAFIs). The RAFI dUY11 is active against otherwise unrelated enveloped viruses, inhibiting entry with no obvious effects on physiological cellular fusions. Using VSV as a model, we had shown that dUY11 targets the lipids in virion envelopes to prevent formation of the negative membrane curvature required for fusion. We now extend these studies to clinically important viruses (HSV-1, -2, HCV, influenza) and another RAFI, aUY11. We analyzed plaquing and focus forming efficiency of virions exposed to aUY11 or dUY11 prior to infection. aUY11 and dUY11 inhibited the infectivity of important human viruses with similar IC50, including HSV-1 (IC50, 131 nM or 48 nM, respectively), HSV-2 (IC50, 47 nM or 49 nM), HCV JFH-1 (IC50, 180 nM or 95 nM) and influenza (IC50, 200 nM or 100 nM). We had tested the fluorescence spectra of dUY11 and aUY11, showing that they localized to the VSV lipid envelope. We now show that their spectra are also most similar when mixed with HSV-1 or HCV virions, or protein-free liposomes. These spectra were distinct from those in aqueous buffer but very similar to those in octanol. RAFIs therefore localize to the lipid envelope of otherwise unrelated enveloped viruses. We next tested the effect of RAFIs on the pH-independent fusion of HSV-1 to Vero cells. HSV-1 labeled at self-quenching concentrations with the membrane dye octadecyl rhodamine chloride (R18) was exposed to dUY11, and then added to Vero cells. Fusion was monitored by fluorescence dequenching. Fluorescence was dequenched by 15% for virions exposed to DMSO vehicle, but by only 7.5% for virions exposed to 50 nM dUY11 (IC50 in plaquing assays). Therefore, dUY11 inhibits pH-independent fusion of HSV-1 envelopes to cell membranes. In conclusion, the RAFIs aUY11 and dUY11 inhibit infectivity of otherwise unrelated enveloped viruses at nanomolar concentrations, and their mechanisms against HSV-1 are fully consistent with those previously found for VSV. These results continue to support our model that RAFIs target virion envelope lipids to prevent fusion of viral and cellular membranes.
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Inhibition of cellular p38 MAP kinase impairs influenza virus induced primary and secondary host gene responses and protects mice from lethal H5N1 infection

Yvonne Boergeling1, Mirco Schmolke1,3, Dorothee Viemann2,4, Johannes Roth2, Stephan Ludwig1
1University of Muenster, Institute of Molecular Virology, Muenster, Germany, 2University of Muenster, Institute of Immunology, Muenster, Germany, 3Present address: Mount Sinai School of Medicine, Department of Microbiology, New York, USA, 4Present address: Department of Pediatric Pulmonology, Allergology and Neonatology, Hannover, Germany

Hidden formatting deleted. Delete this text! mso-ansi-language:EN-US" lang="EN-US">One characteristic of infections with highly pathogenic avian influenza viruses (HPAIV) is the cytokine burst that strongly contributes to viral pathogenicity. It has been suggested, that this hypercytokinemia is an intrinsic feature of infected cells and involves hyperinduction of p38 MAP kinase. Here we investigate the role of p38 signaling in the antiviral response in cells and animals. Global gene expression profiling of HPAIV infected cells in the presence of the p38 inhibitor SB202190 revealed, that inhibition of p38 leads to reduced expression of type I interferons (IFN) and other cytokines after H5N1 and Hidden formatting deleted. Delete this text! Tahoma;color:black" lang="EN-GB">H7N7Hidden formatting deleted. Delete this text! Calibri;mso-bidi-font-family:Tahoma;color:black" lang="EN-GB"> infection. More than 90% of all virus induced genes were either partially or fully dependent on p38. This could be attributed to the fact that p38 inhibition not only affects primary gene expression responses to infection but also impairs the secondary gene expression response by interference with the JAK/STAT pathway. In vivo inhibition of p38 leads to a nearly complete shutdown of virus induced cytokine expression concomitant with reduced viral titers, thereby protecting mice from lethal infection. Hidden formatting deleted. Delete this text! mso-ansi-language:EN-US" lang="EN-US">These observations show, that p38 acts on two levels of the antiviral IFN response: Initially the kinase regulates IFN induction and later p38 controls IFN signaling and thereby expression of IFN-stimulated genes. Thus, inhibition of p38 maybe an antiviral strategy that protects mice from lethal influenza via suppression of overshooting cytokine expression.

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3',5'-di-O-Trityluridine Inhibits Dengue and Yellow Fever Virus Replication In Vitro, Specifically Targeting the Initiation Process of the Viral RNA polymerization

Tine De Burgharaeve1, Suzanne Kaptein1, Barbara Selisko2, Mathy Froeyen1, Michael Jacobs3, Bruno Canard3, Arthur Van Aerschot1, Johan Neyts1
1Rega Institute - KULeuven, Leuven, Belgium, 2Université de la Méditerranée, Marseille, France, 3Royal Free & University College Medical School, London, United Kingdom

The dengue fever virus (DENV) and the yellow fever virus (YFV) are members of the genus flavivirus in the family Flaviviridae. An estimated 50 to 100 million cases of DENV infections occur each year and approximately half a million patients require hospitalization. An effective antiviral treatment or vaccine is not yet available, hence, there is an urgent need for potent and safe inhibitors of DENV replication that ideally have broad-spectrum activity against flaviviruses. Here, we report on the activity of 3',5'-di-O-trityluridine (DiTU) on flavivirus replication. The compound results in a dose-dependent inhibition of (i) DENV- and YFV-induced cytopathic effect (CPE) (ECso values
in the low µmolar range), (ii) virus yield (DENV-1 EC50 = 0.9 ± 0.2 µM; DENV-2 EC50= 1.5 ± 1.2 µM; DENV-3 EC50 = 1.5 ± 0.9 µM; DENV-4 EC50 = 1.1 ± 0.1 µM; YFV-17D EC50= 0.8 ± 0.4 µM) and (iii) viral protein production. Activity was also demonstrated in DENV subgenomic replicons (not encoding the structural viral proteins) (EC50= 3.7 ± 1.9 µM), indicating that the compound inhibits intracellular events of the viral replication cycle. DI6T was shown to inhibit the DENV RNA-dependent RNA polymerase (RdRp) (but not the RdRp of the unrelated Coxackie B virus) in a dose-dependent manner with an EC50 of 6.9 ± 0.7 µM. More in particular and interestingly, depending on the template used, the molecule inhibits the primer-independent de novo initiation process of the viral RNA polymerization. Since DI6T has no free 5', it cannot be converted to a nucleotide and must thus per definition act as a non-nucleoside inhibitor of the flavivirus RdRp. So far, even after 30 consecutive passages we were not able to generate drug-resistant variants, indicating a high barrier to resistance. Understanding the precise mechanism by which 3',5'-di-O-trityluridine inhibits the initiation process of the viral RNA polymerization may allow to design highly specific inhibitors of flavivirus replication.

Oral Session 4: Mini-Symposium: Clinical Development of Antiviral Agents

Chair: Phillip Furman, Ph.D., USA
3:00 - 4:00 pm
Royton Hall AB, 3rd Floor

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SEROPREVALENCE OF DENGUE VIRUS SPECIFIC IgG ANTIBODIES AMONG APPARENTLY HEALTHY INDIVIDUALS IN IBADAN, SOUTH-WESTERN, NIGERIA.

Olufunmilayo G. Oyero
The Polytechnic, Ibadan, Nigeria

Dengue has emerged in recent decades as a worldwide public health problem particularly in the Asia-Pacific and America-Caribbean region. However in Africa, the epidemiology and public health effect of dengue is not well defined. This may be due to the gross under recognition and underreporting of the disease in many African countries. In Nigeria, dengue virus studies were conducted from 1964 to 1968 (virus isolation studies) and in 1977 (sero-epidemiological studies). There was no report of further dengue studies until 2009 when the role of dengue virus in febrile cases was assessed. Therefore this retrospective study was undertaken to review past infections among urban dwellers in one of the four ecological zones surveyed in 1977. This will serve to update the level of the immunity status as well as human exposure to this viral agent in the last 34 years. Five hundred and sixty-four (564) serum samples were collected randomly from the eleven Local Government Areas (LGAs) in Ibadan. The samples were obtained from subjects of 1-60 years of age, in a 1:1.8 male: female ratio. Samples were screened for dengue virus IgG antibodies, using the micro well ELISA dengue kit developed by Diagnostic Automation Inc. (Calabasas, CA, USA). The ELISA technique with 100% sensitivity and 96% specificity revealed an overall prevalence of 64% (360/564) in tested individuals. One hundred and thirty-eight (138) males and 222 females were positive for specific dengue IgG respectively and the percentage of prevalence significantly increases with age. The study showed a higher prevalence of dengue antibodies when compared to the 42% obtained in the 1977 survey. This indicates that an extremely high magnitude of dengue virus infection has been present in the city for a long time, suggesting the possibility of endemicity of the disease in Nigeria. Further studies to determine the clinical spectrum, virus serotypes and vectors involved would be carried out. A joint effort for educating the general population on mosquito control for disease prevention should be established.
Resistance Genotypes in CMX001-201 Clinical Trial for CMV Prophylaxis

Scott Foster, Alice Robertson, George Painter, Susan Godkin, Wendy Painter, Randall Lanier
Chimerix, Inc., Durham, USA

CMX001 is an orally bioavailable lipid conjugate of the FDA approved intravenous drug cidofovir (CDV). The active metabolite, CDV diphosphate, exerts its antiviral effects by acting as a potent alternate substrate inhibitor of viral DNA synthesis. A Phase 2 clinical trial for CMV prophylaxis/preemption in transplant recipients at risk for CMV disease (CMX001-201) has recently been completed. Final results demonstrate that a CMX001 dose of 100 mg twice daily met the primary endpoint, a significant reduction in emergence of CMV disease and/or emergence of viral load > 200 copies/mL at the end of treatment versus placebo. DNA sequencing of the UL54 and UL97 genes was conducted to explore treatment emergent mutations. Among the 171 CMX001-treated subjects, only one known or putative resistance mutation (UL54 R1052C) was detected, appearing in three subjects. The R1052C mutation has been previously reported, in conjunction with V781I, in a single clinical isolate that was resistant to GCV (ganciclovir), CDV, and foscarnet (FOS). V781I confers resistance to GCV and FOS, and therefore it is expected that R1052C confers the CDV resistance. Marker transfer experiments are underway to formally determine whether R1052C confers CMX001 resistance. As expected, no resistance associated mutations were detected in UL97 kinase. The putative role of R1052C in CMX001 clinical resistance is supported by the virologic response to CMX001, CDV and valganciclovir (vGCV). Of the three subjects with R1052C containing virus, one had the variant detected at baseline. This subject did not have a virologic response to CMX001 but had a complete virologic response to subsequent vGCV. The other two subjects had undetectable CMV plasma viremia at baseline, so it was not possible to determine their baseline CMV genotype. Both subjects had the R1052C mutation detected concomitant with viral breakthrough. One of these subjects subsequently initiated CDV therapy while the other initiated vGCV therapy. The subject receiving CDV did not respond, but the subject receiving vGCV had a complete virologic response. These results suggest R1052C is a rare mutation in these subjects that confers resistance to CMX001 and CDV, but does not preclude second line vGCV therapy.

Genotypic Characterization of HCV Variants from the Proof of Concept Study of Miravirsen (MIR), an Oligonucleotide Targeting miR-122, In Treatment Naïve Patients with Genotype 1 (GT1) Chronic HCV Infection

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Background and Aims: MIR is a β-D-oxy- Locked Nucleic Acid modified phosphorothioate anti-sense oligonucleotide inhibitor of the liver-expressed microRNA-122 (miR-122). miR-122 binds to two closely spaced target sites (S1 and S2) in the 5′ untranslated region (UTR) of the HCV genome, and forms an oligomeric miR-122-HCV complex, thereby protecting the 5′ HCV genome from nucleolytic degradation (Machlin et al. PNAS 2011). A third binding site (S3) in the 3′UTR has also been described (Henke, EMBO 2008). In this study we evaluated the development of resistance in patients to MIR following subcutaneous (SC) administration of MIR at doses of 3, 5 and 7 mg/kg in a multiple ascending dose phase 2a trial.

Methods: Patients with chronic HCV GT1 were enrolled sequentially to one of three cohorts (3, 5 and 7 mg/Kg) and followed until week 18. MIR was given as five weekly SC injections over 29 days. Samples for sequence analysis were taken at baseline, end of treatment (week 5), and the first and second visits in which HCV RNA had increased
approximately >1 log_{10} over nadir. Amplification and sequence analysis of miR-122 sites (S1, 2 and 3) was accomplished by site-specific primed RT-PCR followed by population-based sequencing.

Results: MIR was associated with dose-dependent, sustained reductions in HCV RNA that continued after the completion of MIR therapy. No resistance-associated nucleotide changes were observed in S1, S2 and S3 at the end of treatment (wk 5) in any MIR treated patients. In Cohorts 1, 2 and 3, three, five and four MIR subjects experienced HCV RNA rebound (>1 log HCV RNA decline with subsequent >1 log HCV RNA increase). In these twelve subjects no resistance-associated nucleotide changes were observed in S1, S2 and S3 following HCV RNA rebound. Conclusions: MIR, the first microRNA-targeted therapy to be administered to patients, showed continuous and prolonged anti-viral activity well beyond the end of active therapy with no evidence of resistance up to 18 weeks of study. These results provide evidence of MIR's high genetic barrier to resistance.

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Multiple Development Pathways of Pyrimidinediones as Topical Microbicides to Prevent the Transmission of HIV

Karen W Buckheit¹, Anthony Ham¹, Patrick Kiser², Charlene Dezzutti³, Robert W Buckheit, Jr²

¹ImQuest BioSciences, Inc., Frederick, USA, ²University of Utah, Salt Lake City, USA, ³Magee Womens Research Institute, University of Pittsburgh, Pittsburgh, USA

The pyrimidinediones (PYDs) are small molecules which act as highly potent nonnucleoside RT inhibitors at subnanomolar concentrations and inhibit virus entry at nanomolar concentrations. The suppression of two critical early occurring (pre-integration) steps in HIV transmission and infection renders these agents valuable as potential topical microbicides. In vitro, ex vivo and in vivo evaluations have been performed with the lead PYDs (IQP-0528 and IQP-0532) to evaluate their safety and efficacy as microbicides and to optimize their formulation for development in multiple formats. Efficacy and toxicity data in fresh human PBMCs infected with clinical strains of virus demonstrated that the compounds are highly potent and safe microbicides and interact in an additive to synergistic manner with other microbicides in development. Activity of the PYDs in cervical explant challenge models provided additional rationale for continued development of the compounds. The preformulation characteristics of the various PYDs suggest multiple pathways for development of a PYD-containing product formulated as a gel, intravaginal ring, or film, alone or in combination. IND-directed safety and toxicology studies have been completed with our lead PYDs and demonstrate a significant safety margin, suggesting that a long-acting microbicide product containing a PYD will possess attractive pharmacokinetic and pharmacodynamic properties. Like other RT inhibitors, the PYDs may also be used as pre-exposure prophylaxis agents. Data obtained with the congener IQP-0410 demonstrated that the oral bioavailability and pharmacokinetics of the PYDs will yield effective blood and tissue concentrations of the compounds. The PYDs represent novel microbicide candidates based on their mechanism of action, high potency, lack of toxicity, compatible formulation characteristics, and toxicology profile. Three PYDs, IQP-0410, IQP-0528, and IQP-0532 are currently undergoing multiple pathway development in gel, ring, film and PrEP formats.

Oral Session 6: Retroviruses and Herpes Viruses

Chairs: Jose Este, Ph.D. and Debra Quenelle, Ph.D.

1:30 - 3:45 pm

Royton Hall AB, 3rd Floor

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Prodrugs of Acyclic Nucleoside Phosphonates: Novel Approaches
Zlatko Janeba
IOCB AS CR, Prague, Czech Republic

Acyclic nucleoside phosphonates (ANPs) represent a recognized class of antiviral and anticancer agents. ANPs have originated from the successful collaboration between teams of Antonín Holý (Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic) and Erik De Clercq (Rega Institute for Medical Research, K.U. Leuven, Belgium). Later on, substantial number of scientists worldwide became involved in the synthetic and biological studies of various derivatives of ANPs. To overcome high polarity of ANPs at physiological pH and to improve their oral bioavailability, ANPs need to be administered in the form of suitable prodrugs. Diverse types of ANPs prodrugs have been studied and various synthetic transformations of ANPs to the corresponding prodrugs have been developed. Two orally administered ANPs prodrugs (adefovir dipivoxil and tenofovir disoproxil fumarate) have been approved by regulatory agencies worldwide for clinical use (either alone or in combinations) and other promising candidates are in the pipelines (e.g. GS 7340, GS 9191). Recently, we have concentrated the effort of our medicinal chemistry group to significantly improve synthetic approaches towards some commonly used types of ANPs prodrugs, as well as to develop novel types of prodrugs of ANPs. Most recent results on very efficient synthesis and biological evaluation of various symmetrical bis-amidates and mixed mono-amidates of ANPs will be presented. Acknowledgements: Supported by IOCB AS CR (Z40550506) and Ministry of the Interior of the Czech Republic (VG20102015046). References: 1. (a) Holý, A. Curr. Pharm. Des. 2003, 9, 2567–2592. (b) Holý, A. Antiviral Res. 2006, 71, 248–253. (c) De Clercq, E. Antiviral Res. 2007, 75, 1–13. 2. De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. Nature 1986, 323, 464–467. 3. Jansa, P.; Baszczyński, O.; Dračinský, M.; Votruba, I.; Zídek, Z.; Bahador, G.; Stepan, G.; Cihlar, T.; Mackman, R.; Holý, A.; Janeba, Z. Eur. J. Med. Chem. 2011, 46, 3748–3754.

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Screening and Synthesis of Deoxyhypusine Synthase Inhibitors Targeting a Cellular Factor needed in HIV-1 Replication
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Since the first diagnosis of AIDS in 1981 great efforts were made to treat HIV infections. Among the strategies, the introduction of HAART in the mid '90s was an important improvement in combating HIV. HAART drugs primarily target the viral enzymes. However, the occurrence of drug resistance and potential side-effects in long-term HAART require the search for new targets and subsequent development of novel drugs. Various cellular cofactors play an important role in the HIV replication cycle, e.g. the eukaryotic initiation factor 5A (eIF-5A), which is involved in the transport of the unspliced and incompletely spliced viral mRNAs from the nucleus to the cytoplasm. A unique post translational modification of a specific lysine residue to the unusual amino acid hypusine is mandatory for activation of eIF-5A. Two human enzymes are involved in this process: the deoxyhypusine synthase (DHS) and the deoxyhypusine hydroxylase (DOHH). It was shown, that the compound CNI-1493 efficiently inhibits DHS, preventing the activation of eIF-5A and thereby suppressing HIV replication. Recently, we solved the first high resolution crystal structure of DHS in the complex with CNI-1493, demonstrating that CNI-1493 is not an active site inhibitor. To obtain more insights into CNI-1493s structure activity relationship we here present the synthesis, the evaluation of DHS inhibition and the in vitro-inhibitory potency on HIV-1 replication of several active CNI-1493
derivatives. Additionally, we applied structure-based drug design approaches for the development of novel, active site DHS inhibitors and will present the synthesis and the in vitro evaluation of a structurally different new DHS inhibitor that showed marked inhibitory activity against DHS.

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A phenylthiadiazolylideneamine derivative that potently ejects zinc from both retroviral nucleocapsid zinc fingers inactivates HIV virions by destabilizing the viral genomic RNA

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The retroviral nucleocapsid protein of HIV-1 (NCp7) plays multiple roles in viral replication. It interacts with the psi sequence of viral genomic RNA to promote selective encapsidation into viral particles and is also important for efficient proviral DNA synthesis. Inside the infectious virus particle, NCp7 molecules coat and condense the genomic RNA. The NCp7 function relies on specific interactions with nucleic acids, which are mediated by two strictly conserved zinc finger CCHC motifs that bind Zn2+ with high affinity. We have identified 2-methyl-3-phenyl-2H-(1,2,4)thiadiazol-5-ylideneamine (WDO-217) as a new lead molecule that inhibits infections of a wide spectrum of wild-type and drug-resistant HIV-1, HIV-2 and SIV strains, including X4 and R5 HIV-1 strains. Moreover, isolated HIV particles that are pretreated with this compound were unable to infect permissive cells. Whereas capture of virus by DC-SIGN was unaffected by the compound, it efficiently prevented the transmission of DC-SIGN-captured virus to CD4+ T-lymphocytes, suggesting its potential as topical microbicidal component. Mechanism of action studies demonstrated that WDO-217 efficiently ejects zinc from both zinc fingers of the retroviral nucleocapsid protein NCp7 even when bound to oligonucleotides. Interestingly, exposure of isolated virions to WDO-217 reduced the amount of virion-associated genomic RNA as measured by real-time RT-qPCR. This effect on viral genomic RNA stability was also observed with other classes of NCp7 zinc ejectors, underlining the more general effect on viral genomic RNA when unfolding NCp7. The discovery of this new lead compound and the novel mechanism of action open opportunities for the development of new series of zinc ejectors as candidate topical microbicidal agents.

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Compensatory mutations rescued the virus replicative capacity of VIRIP resistant and VIR576 cross-resistant HIV-1

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VIRIP has been identified as a natural inhibitor of HIV-1 that blocks HIV-1 gp41-dependent fusion. VIR576, an analogue of VIRIP has been reported the effectivity of VIR576 in a phase I/II clinical trial. We have shown a high genetic barrier for resistance due to 7 mutations in both gp120 and gp41. Here, viruses isolated at different stages of the generation of the VIRIP-resistant virus were evaluated for their replicative capacity in lymphoid cells. Proviral DNA was used to identify mutations conferring resistance or replicative capacity. Drug-susceptibility of single drugs and drug combinations was measured in a standard MT-4/MTT assay. Wild type HIV-1 (NL4-3 and HxB2) was susceptible to the VIRIP analogue VIR576 with 50% effective concentrations of 0.3 mM. Three mutations (2 in gp120: A433T/V489I and one in gp41: V570I) were sufficient to confer resistance to VIRIP and its analogues but reduced virus replicative capacity by 8-fold (p<0.01). However, accumulation of additional
mutations in both gp120 and gp41 rescued virus fitness while retaining resistance to VIR576 but complete sensitivity to T20. Combinations of VIR353 with T20 or AZT showed an additive effect when tested against wild-type HIV-1 NL4-3. Our results indicate a similar mode of action between VIRIP and VIR576 and a role for gp120 in the anti-HIV activity of VIRIP analogues. VIRIP-resistance comes with a high cost to viral fitness that is compensated by additional mutations in both gp120 and gp41. Drug combinations suggest that T20 and VIR353 do not interfere with each other in their binding to HIV-1 gp41. Our results provide additional support to the development of this new class fusion inhibitors as antiretroviral agents.

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Anti-HIV-1 gene therapy using autologous CD4+ T cells modified with a retroviral vector expressing a bacterial endoribonuclease MazF

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HIV-1 infection is still an incurable disease, although a number of effective antiretroviral drugs have been approved for treatment of patients. Combination chemotherapy with these drugs reduces the viral load to undetectable levels, however their adherence problem and long-term toxicity clearly indicate the need for additional treatment strategies. We hypothesize that genetic modification conferring resistance to HIV-1 replication on CD4+ T cells may delay disease progression by reducing the viral load and maintaining sufficient CD4 count. Here, we proposed to use the Escherichia coli gene mazF as a payload. MazF is an endoribonuclease that cleaves RNA at conserved ACA sequences. It has been reported that over expression of MazF cleaves mRNA but not ribosomal and transfer RNA. Since HIV-1 RNA has more than 240 ACA sequences in its genome, it is assumed that the viral RNA is highly susceptible to the nuclease activity of MazF. Therefore, we constructed a γ-retroviral vector in which mazF was fused to the downstream of HIV-1 LTR, so that MazF is expressed conditionally only in HIV-1-infected cells undergoing viral replication. CD4+ T cells introducing mazF (MazF-T cells) restricted HIV-1 replication in vitro. The viability of MazF-T cells was not affected probably due to the consequence of either a low level or a short period of MazF induction. The expression of Tat protein was kept low in MazF-T cells even after HIV-1 infection, suggesting that Tat mRNA is also a target of MazF and that the low level of Tat expression is a limiting factor for MazF induction. The Tat-dependant MazF induction leads to suppression of HIV-1 RNA synthesis more efficiently than that of cellular mRNA species forming complex with ribosomes or an unknown mechanism. Indeed, cellular mRNA of MazF-T cells was not affected by HIV-1 infection, even when HIV-1 RNA was significantly reduced. Taken together, we conclude that MazF-T cells gain the function of controlling HIV-1 replication and that our strategy could be extended to the treatment of HIV-1-infected patients.

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Sensitivity of Clinical Isolates to Helicase Primase Inhibitors and No Detection of Resistance Mutations above Background Frequency

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AIC316 (BAY 57-1293) is an HSV helicase-primase inhibitor (HPI) that has recently undergone a successful phase 2 trial against genital herpes. The aim of this study was to characterize a collection of HSV-1 and HSV-2 clinical isolates obtained in the US several years before any HPI had been tested in the clinic. Isolates were screened for sensitivity to AIC316. In a classical plaque-reduction assay (PRA) all of the 27 HSV-1 and 32 HSV-2 isolates were sensitive to AIC316 with a mean EC50 of 0.026 and 0.030 μM, respectively. A higher titre (10^6 p.f.u.) was used to screen for presence of HPI-resistant mutants. A frequency of approx. 10^-6 is assumed to be in the range of spontaneous mutation. 19 of 27 HSV-1 isolates (70%) but only 8 of 32 HSV-2 isolates (25%) yielded plaques in this range. 17 of the HSV-1 plaques had a substitution of K356 in UL5 (helicase), previously identified as a site important for sensitivity to HPI. All were also cross-resistant to an alternative HPI, ASP2151. 3 plaques had no change in UL5 but contained a substitution in the UL52 primase gene (A899T) these were sensitive to ASP2151. Several of the HSV-2 plaques had mutations at residue UL5 K355 (homologous to K356 in HSV-1). 1 HSV-2 isolate (US2/22) derived from an immunocompromised patient had UL5 G351R substitution at approx. 10^4 however, when drug-sensitive US2/22 was plaque-purified, the frequency of resistant mutants dropped to background. A spontaneous plaque from plaque-purified US2/22 showed normal UL5 but a three-base deletion (∆) in UL52 corresponding to 1 of the 3 consecutive alanines at position 903-905 (A905 is homologous to A899 in HSV-1). Interestingly, in contrast to HSV-1 UL52 A899T, the HSV-2 ∆A905 mutant is cross-resistant to ASP2151. In summary, all clinical US isolates were fully sensitive to HPI and, except for one isolate from an immunocompromised patient, all had resistant plaques at background frequency with a trend to lower variability for HSV-2 compared to HSV-1.

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Excellent Efficacy and Pharmacokinetics have been Demonstrated in Pre-clinical and Phase I/II Studies by AIC316, a Novel Drug Against Herpes Simplex Virus (HSV) Type 1 and 2

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HSV infections still cause considerable morbidity. Many individuals suffer painful recurrences, often associated with severe psychological distress. Infections in newborns or immunocompromised can be life-threatening. Transmission of HSV-2 has become a major health concern since it also promotes transmission of other sexually transmitted diseases, e.g. HIV. Nucleoside analogs are widely used for treatment but recurrences still occur after cessation of therapy and even under long-term treatment, and transmission of HSV-2 cannot be prevented efficiently. AIC316 belongs to a new class of antiviral compounds against HSV. In contrast to nucleoside analogs targeting the viral DNA polymerase AIC316 prevents the de novo synthesis of viral DNA through inhibition of the viral helicase-primase complex. AIC316 does not require activation by viral thymidine kinase. In vitro and in vivo, AIC316 exhibited potent and rapid antiviral activity as well as superior efficacy against both HSV-1 and -2 compared to nucleoside analogs. In single and multiple dose phase I trials AIC316 was safe with favorable pharmacokinetics resulting in a long half-life, indicative of efficacy with once per day dosing. In a recently completed phase II proof-of-concept and dose finding trial in subjects with genital herpes AIC316 showed excellent activity by reduction of viral shedding in a dose dependent way. Similarly, clinical symptoms were suppressed in a
dose dependent manner, even with once weekly dosing. Phase III clinical trials are currently in preparation. In conclusion, AIC316 represents a highly active and novel treatment option for HSV-1 and -2 infections with very convenient dosing.

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A three-year experience of a translational research platform for the evaluation of drug-resistance among Herpesviruses
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Background. Drug-resistance in herpesviruses is virtually not observed in immunocompetent patients; however, it is a well-recognized problem in the immunocompromised host. Therefore, a Reference and Service Center named RegaVir [Research Group for Antiviral Resistance, (www.regavir.org)] for the diagnosis and typing of drug-resistant herpesviruses has been established in Belgium in January 2009, supported by the Belgian National Cancer Plan.

Methods. Phenotyping (drug-susceptibility profile in human embryonic lung fibroblasts) or genotyping (PCR amplification of viral genes involved in drug-resistance [UL97 (protein kinase) and UL54 (DNA polymerase) for HCMV; UL23 (thymidine kinase) and UL30 (DNA polymerase) for HSV, ORF36 (thymidine kinase) and ORF28 (DNA polymerase) for VZV and U69 (protein kinase) and U38 (DNA polymerase) for HHV-6], followed by DNA sequencing) were performed according to the virus and the type of sample received. Results. Between January 2009 and February 2012, a total of 322 samples (recovered from patients that were refractory to antiviral therapy) were received by RegaVir for performing phenotyping and/or genotyping. Our results showed: a) a considerable number of isolates bearing mutations linked to drug-resistance [65.2% (30/46) for HSV-1, 75.9% (44/58) for HSV-2, 32% (8/25) for VZV, 28.7% (37/129) for HCMV and 50% (2/4) for HHV-6] among the samples that proved positive for virus isolation and/or PCR amplification, b) the identification of unknown genetic polymorphisms and of novel mutations most likely linked to drug-resistance, c) a higher risk for developing drug-resistance in the central nervous system, d) multiple drug-resistant herpesviruses conferred by infection with multiple viral strains, e) compartmentalization of drug-resistant herpesviruses, f) the need to extend the present platform to other viruses such as adenovirus and polyomavirus. Conclusions. Rapid genotyping and/or phenotyping of herpesvirus isolates recovered from immunocompromised individuals refractory to antiviral therapy is recommended for the adjustment of antiviral therapy.

Poster Session 1: Retroviruses, Hepatitis Virus, Respiratory Viruses, Emerging Viruses and Antiviral Methods

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A novel peptide derived from measles virus fusion protein inhibits the replication of subacute sclerosing panencephalitis (SSPE) virus in vitro and in vivo.
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[Background] Subacute sclerosing panencephalitis (SSPE) is a progressive and fatal central nervous system disorder by a measles virus variant (SSPE virus). Novel therapeutic strategies are urgently required. The fusion protein (F protein) plays an important role in the expansion of infection, and contains two critical heptad repeat (HR) regions (HR1 and HR2). In HIV-1, enfuvirtide, a synthetic peptide derived from the HR region of the fusion protein (gp-41), inhibits viral replication and is utilized as an anti-HIV agent in patients. The HR regions of HIV-1 gp41 are structurally and functionally similar to those of the measles virus F protein. [Methods] Three peptides, M1, M2, M2EK, were synthesized. M1 is referenced from the previous report (Proc. Natl. Acad. Sci. USA, 93, 2186-2191, 1996). M2 and M2EK are novel peptides. M2EK is based on M2, in which the α-helix structure is stabilized by the structural addition of EK. Vero cells and Vero/SLAM cells were used in all experiments. Measles virus (Edmonston strain) and SSPE virus (Yamagata-1 strain) were used. The EC_{50} were determined by viral plaque reduction assay. The CC_{50} were obtained by MTT test. The antiviral activity of the peptides in vivo was evaluated in nude mice infected with SSPE virus. Enfuirtide was used as a control peptide. [Results] The EC_{50} values of M1, M2 and M2EK against the Edmonston strain were 27.3±7.5, 124.4±38.9 and 101.1±70.9 (nM mean±S.D.), respectively. The EC_{50} values of M1, M2 and M2EK against the Yamagata-1 strain were 13.3±7.9, 265.0±167.9 and 49.0±32.6 (nM mean±S.D.), respectively. The CC_{50} values were more than 100μM for all peptides. Intracranial administration of M2EK in nude mice tended to increase the survival rate compared with M1-treated and untreated mice (p=0.005). [Conclusions] A novel peptide, M2EK, derived from the HR2 region of the F protein inhibits the replication of SSPE virus both in vitro and in vivo.

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Generation of dengue virus resistant to carbohydrate-binding agents results in the deletion of the unique N-glycosylation sites on the viral envelope E-glycoprotein

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Dengue virus (DENV) is one of the most important emerging viruses in the world. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is present on the dendritic cells (DC) in the skin, the first target cells. DC-SIGN recognizes N-glycosylation sites on the viral envelope of several viruses. We evaluated the antiviral activity of carbohydrate-binding agents (CBAs), such as the plant lectins HHA, GNA and UDA in Raji/DC-SIGN+ cells and monocyte-derived DC (MDDC) and demonstrated a consistent and serotype-independent anti-DENV activity. Recently, we generated a HHA-resistant DENV in mosquito cells. The E-protein contains 2 N-glycosylation sites, which were mutated in HHA^{res} virus (N67D and T155I), indicating that HHA specifically interacts with these glycans. HHA^{res} virus was not able to infect Raji/DC-SIGN+ cells and also not MDDC, demonstrating the crucial carbohydrate-dependency in DC-SIGN-mediated viral infection. This mutant virus was used to identify the antiviral target of other compounds as it could replicate in human liver Huh-7 cells. HHA^{res} DENV was cross-resistant to GNA, recognizing α-1,3 and α-1,6 mannose residues. UDA, recognizing N-acetylglucosamine residues, lacked antiviral activity against HHA^{res} DENV. Pradimicin-S (PRM-S), a small-size α-1,2-mannose-specific CBA, was unable to inhibit HHA^{res} DENV, demonstrating that PRM-S targets the glycans on the DENV envelope. In contrast, ribavirin, a nucleoside analogue, retained its antiviral activity against wild-type as against HHA^{res} virus, indicating no compensatory mutations in the non-structural proteins of DENV. A novel doxorubicine analogue, SA-17, which interferes with the DENV entry process, was equipotent active against wild-type and HHA^{res} DENV. SA-17 is predicted to interact with the hydrophobic binding pocket of the E-protein independently from the N-glycosylation state of the E-glycoprotein. In conclusion, we generated a HHA^{res} DENV-2
strain that is not able to infect its target cells through DC-SIGN. HHA′res DENV can be a useful tool to elucidate the specific targets of novel viral entry inhibitors.

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Labyrinthopeptins, a new class of lantibiotics, exhibit potent anti-dengue virus activity

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The labyrinthopeptins are a new class of lantibiotics containing two identical quaternary amino acids, named labionin. The synthetic formation of this unique structural feature represents the key step in the total synthesis of these polycyclic peptides. Various bioactivity assays revealed antiviral activity against herpes simplex virus (HSV) and reduction of neuropathic pain in a mouse model. We evaluated the antiviral activity of two labyrinthopeptins (named Laby A1 and Laby A2) against dengue virus (DENV) which is one of the most important emerging viruses in the world. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) is present on dendritic cells (DC) and is described to be an important cellular receptor for DENV to enter and infect human cells. Because DC in the skin are the first target cells for DENV after a mosquito bite, we used in addition to the DC-SIGN-transfected Raji cell line, monocyte-derived DC (MDDC) generated from human blood resembling the real target cells for viral infection. We observed with Laby A1 a consistent dose-dependent inhibition of replication of all four serotypes of DENV in Raji/DC-SIGN+ cells and in MDDC (EC50: 0.6 - 2.3 μg/ml). However, Laby A2 had only weak, if any, antiviral activity (EC50: 7.7 - > 50 μg/ml). Binding studies revealed that Laby A1 interacts with DENV and not with cellular DC-SIGN. When Laby A1 was added after DENV was bound to the cells, the peptide was not able anymore in inhibiting viral infection. This indicates that Laby A1 acts at an early step in the viral replication cycle. We were able to generate a mutant virus lacking both N-glycosylation sites on the viral E-glycoprotein. The mutant virus could not infect DC-SIGN- cells anymore, in contrast to DC-SIGN+ cells (such as liver Huh-7 cells) which could still be infected with the mutant virus. As Laby A1 is equipotent against the wild type virus and the N-glycan deleted virus, it indicates that Laby A1 does not interfere with the N-glycans but interacts in a different way with the DENV E-glycoprotein.

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A Novel Oxatricyclic-ligand-Containing Nonpeptidic Protease Inhibitor (PI) GRL-0519A Potent against Multi-PI-Resistant HIV In Vitro.

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[Background] We identified GRL-0519A (519), a novel PI, containing the structure-based-designed privileged non-peptide oxatricyclic P2-ligand, tris-tetrahydropyranylurethane (tris-THF). Here, we demonstrate its anti-HIV activity and cytotoxicity in vitro. [Methods] The activity of 519 and FDA-approved PIs against wild-type HIV-1, HIV-2, and drug-resistant HIV-1 was determined using MTT or p24 assays using MT-2, MT-4, or PHA-PBM as target cells. For
the selection of PI-resistant HIV-1 variants, MT-4 cells were exposed to HIV-1_{NL4-3} or a mixture of 8 highly multi-drug-resistant clinical isolates (HIV_{MDR}) and cultured in the presence of increasing concentrations of each PI. Effects of human serum proteins on 519’s antiviral activity were examined employing human serum albumin or alpha-1-acid glycoprotein (AAG). Molecular interactions between 519 and HIV-1 protease (PR) were examined with crystallographic analysis. [Results] 519 was highly potent against HIV-1_{LA} (EC\textsubscript{50}: ~0.7 nM) and HIV-2 with minimal cytotoxicity (CC\textsubscript{50} ~44,600 nM). 519 blocked the replication of each of HIV-1_{NL4-3} variants selected with 5 μM of atazanavir, lopinavir, or ritonavir (EC\textsubscript{50}: 2.8 – 3.3 nM), and also maintained its activity against highly darunavir (DRV)-resistant variants (EC\textsubscript{50}: 5.6 – 30.0 nM). 519 was potent against 6 multi-drug-resistant variants with EC\textsubscript{50} values ranging from 0.9 to 4.3 nM. The effects of human serum protein binding on 519’s antiviral activity were insignificant. Structural analyses revealed that 1st and 2nd THF of 519 have tight hydrogen binding with the PR active site Asp29 and Asp30. In addition, 3rd THF of 519 has interactions with pleural amino acids of the PR flap, catalytic core, and dimer-interface. The development of resistance against 519 was substantially delayed compared to other control PIs including amprenavir, tipranavir, or DRV both in HIV-1_{NL4-3} and the HIV-1_{MDR} mixture used as a starting strain. [Conclusions] The present data suggest that 519 serves as a favorable candidate as an agent for treating individuals harboring multi-PI-resistant HIV.

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**GRL-01511A : A novel HIV-1 protease inhibitor potent against multi-PI-resistant HIV-1s**

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**Background:** Currently available antiretroviral therapy for HIV-1 infection and AIDS potently suppresses the replication of HIV-1 and significantly extends the life expectancy of HIV-1-infected individuals. However, there exist multiple challenges in treating HIV-1/AIDS over extended years, including the inability to wipe out HIV-1 in infected individuals and the emergence of multi-drug-resistant HIV-1 (HIV-1_{MDR}) variants. Continuous efforts are required to develop more potent and safer therapeutics with high genetic barrier. We identified a non-peptidic HIV-1 protease inhibitor (PI), GRL-01511A (015), and here demonstrate its anti-HIV-1 activity and cytotoxicity in vitro. **Methods:** Anti-HIV-1 activity of newly synthesized compounds was determined with MTT and p24 production inhibition assays using MT-2 or MT-4 cells. The intermolecular fluorescence resonance energy transfer (FRET)-based HIV-1 expression assay was used to determine the activity of selected compounds to block protease dimerization. HIV-1 variants were generated by propagating HIV-1 in the presence of increasing concentrations of a compound in MT-4 cells using wild-type or a mixture of 11 multi-PI-resistant HIV-1s (HIV_{mPI}). **Results:** 015 exerted potent activity against wild-type HIV-1_{LA} with an IC\textsubscript{50} value of ~3 nM and minimal cytotoxicity (CC\textsubscript{50} ~80 mM). The compound blocked the replication of highly PI-resistant HIV-1 variants, which had been generated in vitro using the dose-escalation method with each of currently available PIs (amprenavir, lopinavir, atazanavir, tipranavir, and darunavir) and also showed potent activity against 7 recombinant clinical HIV-1 isolates, which were obtained from patients harboring HIV-1_{MDR} variants that showed high-level resistance to most of the FDA-approved PIs. Moreover, 015 had an inhibition activity of dimerization of HIV-1 protease. **Conclusion:** 015 suppresses the replication of HIV_{mPI} more broadly than any of the FDA-approved PIs including darunavir in vitro. It is warranted that 015 be further studied as a possible therapeutic agent for treating individuals harboring wild-type and/or HIV-1_{MDR}.

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**Mutations in NSSA-ISDR in non-responders of combination therapy of HCV 3a infected Pakistani patients**
NS5A-ISDR (Interferon sensitivity determining region) mutations with response to interferon-therapy in the case of Hepatitis C virus (HCV) 1b have been correlated but no such correlation was found in the case of HCV subtypes 3b and 1a. So we decided to study these mutations in genotype 3a of Pakistani patients. In this study, chronic HCV infected Pakistani patients having genotype 3a received Interferon alpha and Ribavirin combinational therapy for six months. Viral loads were performed before and after the treatment. NS5A-ISDR region of HCV was amplified by specific primers followed by sequencing. Sequences were analysed for mutations. Out of total 40 patients, 37 patients completed the therapy. Among them 26 (70%) were end of treatment responders. 11 (30%) patients were virological non-responders. Among non-responders three isolates (3/11) 27.2% had intermediate mutations within the NS5A-ISDR region when compared to HCV-K3a prototype. There was no such relation found between mutations in NS5A-ISDR and response to antiviral therapy. This study cannot be conclusive and needs to be investigated further.

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Inhibition of the HIV-1 Rev-mediated mRNA export pathway by small molecules prevents viral replication
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The HIV-1 Rev protein is an essential regulator for viral replication in that it promotes the nuclear-cytoplasmic transport of late viral mRNAs. Nuclear export of Rev is mediated by its leucine-rich nuclear export signal (NES), which is known to interact with the cellular transport factor or karyopherin CRM1 (XPO1 or exportin-1). CRM1 on its turn forms a ternary complex with RanGTP and directs the Rev-mRNA complex to the cytoplasm where the complex is dissociated and the exported late viral mRNAs serve as templates for viral structural protein synthesis or as viral genome. The interaction of Rev with the cellular co-factor CRM1 is therefore essential for the export of late viral mRNAs and for viral replication, and warrants the consideration of disrupting this process for therapeutic anti-HIV strategies. We have identified small-molecule inhibitors (KPT-185, KPT-251) of the Rev-CRM1 protein-protein interaction. Mechanism of action studies demonstrate that the compounds inhibit the nuclear export of Rev and traps the protein in the nucleus of the HIV-infected cell. The compounds interfere with the viral replication of both wild-type and drug-resistant HIV-1 strains and also suppress the activation of latent infection at submicromolar concentrations and showed low toxicity in normal cells. Using co-immunoprecipitation and co-localization assays we could demonstrate that the compounds dissociate the Rev-CRM1 interaction. Their unique mode of action and broad-spectrum anti-HIV-1 activity makes them valuable leads for the design of future generations of HIV inhibitors. Moreover, they can be investigated for the treatment of other viral infections involving CRM1-mediated transport. Our findings again demonstrate the potential of targeting viral-cellular cofactor protein-protein interactions for the development of novel therapeutic antiviral strategies.

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IND-Directed Development of Dual-Acting Pyrimidinediones IQP-0410 and IQP-0528: Enhancement of Solubility and Metabolic Stability
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The pyrimidinediones are highly potent nonnucleoside inhibitors of both HIV-1 and HIV-2, inhibiting HIV-1 RT at subnanomolar concentrations and the entry of both HIV-1 and HIV-2 at nanomolar concentrations, including all HIV subtypes (except subtype O) and MDR strains. The primary development hurdles associated with IQP-0410 (solubility and metabolic stability with rapid metabolism in both human liver microsomes and human hepatocytes) have been chemically addressed and a new lead (IQP-0528) has been identified and evaluated. IQP-0410 and IQP-0528 were well tolerated in animals with no test article-related findings noted from in-life data, clinical pathology, necropsy or histology. Pharmacokinetic studies demonstrated oral bioavailability with effective concentrations (EC_{50}) of the compounds exceeded by 30-50-fold at 24 hours. Safety pharmacology studies showed no signs of pharmacologic or toxicologic activity and all genotoxicology testing was negative. We have defined two different major pathways of metabolism and new compounds were synthesized to block metabolic degradation. The N_{2\text{-}} cyclopentenyl group in IQP-0410 undergoes multiple oxidations modification of this substituent to N_{2\text{-}}cyclopropyl (IQP-0528) yielded significantly greater stability without loss of antiviral efficacy or enhanced toxicity. With IQP-0528 the major metabolic pathway is oxidation of one of the methyls of the C6-linked C_{6}H_{4}Me_{2} group, as has been reported for the metabolism of other uracil-based NNRTIs, such as Emivirine (MK-442). Replacement of the 3,5-dimethylphenyl with 3,5-dichloro, resulted in molecules which were less active than the parent. IQP-0528 is now being developed as a lead product for both HIV therapy and prevention. Our chemical modification efforts suggest a strategy to develop analogs with improved solubility and reduced metabolism via additional modifications to the ‘right hand side’ of the molecule, including (1) changes at C5 which will improve solubility and oral absorption, (2) decreasing the ClogP of the compounds to decrease metabolism, and (3) decrease metabolism in the C6 chain by introducing electron withdrawing groups to the aromatic ring.

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PD 404,182 is a virucidal small molecule that disrupts hepatitis C virus and human immunodeficiency virus

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We describe a virucidal small molecule, PD 404,182, effective against hepatitis C virus (HCV) and human immunodeficiency virus (HIV). The median IC_{50} values for the antiviral effect of PD 404,182 against HCV and HIV in cell culture are 11 micromolar and 1 micromolar, respectively. The antiviral activity of PD 404,182 is due to physical disruption of virions that is accompanied to varying degrees (depending on the virus and exposure temperature/time) by release of viral nucleic acids into the surrounding medium. PD 404,182 does not directly lyse liposomal membranes even after extended exposure and shows no attenuation in antiviral activity when pre-incubated with liposomes of various lipid compositions, suggesting that the compound inactivates viruses through interaction with a non-lipid structural component of the virus. The virucidal activity of PD 404,182 appears to be virus-specific as little to no viral inactivation was detected with the enveloped Dengue and Sindbis viruses. PD 404,182 effectively inactivates a broad range of primary isolates of HIV-1 as well as HIV-2 and simian immunodeficiency virus (SIV), and does not exhibit significant cytotoxicity with multiple human cell lines \textit{in vitro} (CC_{50} > 300 micromolar). The compound is fully active in cervical fluids although exhibiting decreased potency in the presence of human serum, retains its full antiviral potency for over 8 h when in contact with cells and is effective against both cell-free and cell-associated HIV. These qualities make PD 404,182 an attractive candidate as an anti-HIV microbicide for the prevention of HIV transmission through sexual intercourse.
Liver X receptors agonists impede Hepatitis C virus infection in an Idol-dependent manner

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Hepatitis C virus (HCV) is a major human pathogen that causes many serious diseases, including acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma. Treatments for this virus are inadequate, and improved antiviral therapies are necessary. Although the precise mechanisms regulating HCV entry into hepatic cells are still unknown, the low-density lipoprotein receptor (LDLR) has been shown to be essential for entry of infectious HCV particles. Liver X receptors (LXR) were recently reported to control LDLR expression through the regulation of the expression of the Idol (inducible degrader of the LDLR) protein, which could trigger the ubiquitination and degradation of LDLR. In this study, we analyzed the antiviral effect of Idol in vitro. The results demonstrated that Huh7.5.1 cells that exogenously expressed Idol were resistant to HCV infection. Next, the treatment of HCV-infected Huh7.5.1 cells with either synthetic LXR agonists (GW3965 or T0901317) or the natural LXR ligand 24(S),25-Epoxycholesterol inhibited HCV infection in a dose-dependent manner. Furthermore, a combination of LXR agonists and HCV RNA replication inhibitors exerted additive effects against HCV, as revealed by isobologram analysis. In conclusion, our data indicate that molecules such as LXR agonists, which could stimulate the expression of Idol, represent a new class of potential anti-HCV compounds, and these compounds could be developed for therapeutic use against HCV infection, either as a monotherapy, or in combination with other anti-HCV drugs.

A mechanism of anti-influenza A virus infection mediated by curcumin

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Curcumin (a natural compound in curry) has been shown to exert anti-inflammatory, antioxidant, and anticarcinogenesis properties by modulation of signal transduction pathways including NF-kB, which is required for efficient replication of influenza virus. Our previous observation indicated that curcumin efficiently blocked influenza replication via inhibition of viral haemagglutination (HA) activity. In the present study we showed in addition to influenza virus, pre-treatment of curcumin blocked the plaque formation of other enveloped viruses, whereas enterovirus EV71, a non-enveloped virus, remained unaffected. The effect of curcumin on envelop was determined by liposome-based assays. Incubation of curcumin with the mixture of DNA and liposome decreased the transfection efficiency of Cellfectin™. Moreover, leakage of fluorescence dye was detected when liposome was treated with curcumin. Based on the results of biochemistry analysis, we proposed versatile roles that curcumin plays in inhibition of influenza virus infection.
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Importance of Crimean Congo Haemorrhagic Fever in Iran as an emerging infectious disease
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Background: Crimean Congo Haemorrhagic Fever (CCHF) is a viral zoonotic disease transmitted by CCHF virus belonging to Nairovirus genus and Bunyaviridae family causing a fatal haemorrhagic fever in humans with up to 50% mortality rate. The disease can affect humans by infected tick bite, handling of infected livestock or human blood or tissues and nosocomially. In 1999, after emerging of CCHF in Iran, it was considered as a major public health problem. So we established Arboviruses and Viral Hemorrhagic Fevers Laboratory as a National Reference laboratory in Pasteur Institute of Iran. Methods: From June 2000 to 7 January 2012, 2499 sera samples from probable patients have been collected from different parts of Iran and analyzed serologically (IgM & IgG Elisa technique) and molecularly (gel based and Real time RT-PCR) for CCHF disease in our Lab. Results: Among the 2499 probable sera, we confirmed the disease of 870 cases (126 deaths). The disease has been seen in the majority of Iran provinces (26 out of 31) and has been continuously seen in Sistan-va-Baluchestan province in the last 11 years, which is near to Afghanistan and Pakistan (endemic area), while it was intermittently in the other provinces. Infected patients have high risk professions, such as slaughter house worker, slaughterer, butcher and farmer. Our phylogenetic study showed that the genome isolates from different parts of Iran have close relationship to Matin (Pakistan) strain and also recently, we found an isolate from central part of Iran which is near to Iraqi strain. Conclusion: CCHF is one of the most important viral emerging zoonotic diseases in Iran and Middle East, so it seems that public awareness is very important for disease controlling specially in high risk groups, such as slaughterer, butchers and slaughter house workers. Hence, it is very important to have continuous training program for these high risk groups. With more phylogenetic studies, it is possible to find more strains of CCHF in Iran in addition to Matin (Pakistan) strain and Iraqi strain.

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Enzymatic Characterization of De Novo RNA synthesis using Full-length Dengue Virus RNA-dependent RNA Polymerase
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Dengue virus (DENV) belongs to the family of Flaviviridae and is the most prevalent arthropod transmitted infectious disease in humans. The genome of dengue virus consists of a positive single-stranded RNA with a cap 1 structure $\text{Me}^\text{G}\text{p}p\text{pA}_\text{5'cap}$. Synthesis of the dengue viral RNA is performed by the DENV NS5 RNA-dependent RNA polymerase (RdRp) which is the largest and most conserved protein across the Flavivirus family. It is essential for viral replication and hence represents an attractive target for anti-viral therapy. It catalyzes de novo replication of the viral genome which consists of 3 steps: initiation, transition and elongation. By using a purified full-length dengue NS5 and a RNA template containing the 5' and 3' untranslated region (UTR) of the viral genome, we have determined the optimal enzymatic condition for de novo RNA synthesis. RNA synthesis is greatly enhanced in the presence of MnCl₂. Steady state kinetic parameters were determined for both RNA and NTPs substrates. High concentration of ATP/GTP or pre-incubation with ATP/GTP does not enhance RNA synthesis. High MnCl₂ and low NTP concentration promote terminal transferase activity. Several C-terminal truncated NS5 proteins were cloned
and expressed. RNA synthesis was reduced in some of the truncated proteins, suggesting the C-terminal is important for de novo activity.

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The Green Tea Polyphenol Epigallocatechin Gallate Inhibits Infectivity of Unrelated Enveloped Viruses by Preventing Primary Attachment of Virions to Cells

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Epigallocatechin gallate (EGCG) is the most abundant catechin in green tea. EGCG is active against viruses such as influenza and HIV-1, but its mechanisms of action remain unclear. We now analyzed the effects of EGCG on other viruses, using plaquing and focus forming efficiency assays, in which virions were exposed to EGCG prior to infection of appropriate cells. EGCG inhibited the infectivity of important RNA and DNA human viruses, including HCV JFH-1 (IC₅₀, 1.45 μM), influenza (IC₅₀, 10.1 μM), HSV-1 (IC₅₀, 0.24 μM), HSV-2 (IC₅₀, 0.15 μM), and vaccinia virus (IC₅₀, 1.65 μM). EGCG also inhibited the infectivity of three model viruses, VSV (IC₅₀, 0.99 μM), Sindbis virus (IC₅₀, 10.5 μM) and mCMV (IC₅₀, 0.92 μM). Using VSV and HSV-1 as models, which enter through pH-dependent or independent mechanisms, respectively, we tested the antiviral efficacy of EGCG. EGCG acted directly on the virions, and not on the target cells. VSV and HSV-1 virions labeled at self-quenching concentrations with the membrane dye octadecyl rhodamine chloride (R18) were exposed to EGCG for 10 minutes at 37°C. There was no dequenching of R18 fluorescence under these conditions, whereas lysis with Triton X-100 resulted in the expected dequenching. EGCG therefore does not lyse virions. In contrast, EGCG inhibited virion attachment. ³⁵S-labeled HCV, HSV-1 or VSV virions pre-exposed to EGCG were adsorbed onto cells at 4°C before washing away the unattached virions and measuring the radioactivity attached to cells. EGCG inhibited attachment of HCV (IC₅₀, 37.7 μM), HSV-1 (IC₅₀, 15.2 μM) or VSV (IC₅₀, 24.9 μM). We also developed an efficient non-radioactive binding assay. R18-labeled HSV-1 or VSV virions were treated with EGCG prior to adsorption onto cells at 4°C. Unattached virions were washed away, and the R18 fluorescence still attached to cells after the washes was measured. Attachment of R18-labeled HSV-1 or VSV was inhibited in this assay with most similar IC₅₀ (9.9 μM or 18.7 μM, respectively) to those in the conventional ³⁵S binding assays. In conclusion, EGCG inhibits infectivity of enveloped viruses by preventing the primary attachment of virions to cells, without lysing the virions.

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Action mechanism of the anti-influenza virus active Kampo (Traditional Japanese herbal) medicine, Hochuekkito

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When Kampo medicine, Hochuekkito (Hochu) was administered for two weeks to normal mice, influenza virus was subsequently reduced. The action mechanism of Hochu was examined using the plaque assay method et.al. The possibility of obstructing the first stage of the infectious process (adsorption and entry) and the direct possibility of action against virus particles were suggested. In the plaque assay method, individual effects on both could not be identified. Virus RNA in the infective cell was verified by quantitative RT-PCR. An equal inhibition effect was obtained when Hochu was preprocessed for normal cells and when they were made to act simultaneously with virus adsorption. The viral load that attaches to the surface of the cell when the virus particle is inert by UV irradiation increased in the Hochu-administered group. Moreover, the affinity of Hochu and virions was hundreds
of times as higher than affinity with the host cell. The effect of entry obstruction by Hochu became clear in image analysis on the amount of virus nucleocapsid protein (NP) invading the cell that detected the FITC-labeled NP antibody. Moreover, it seems not to act on the nucleic acid synthesis system, virion release to outer cells, and subsequent second round infection in the infectious process. In conclusion, Hochu forms virus particles and complex or can obstruct the entry of influenza virus infection into cells. Although we cannot be described about the preventive effect of a mouse directly, it is clear from these results that Hochu is effective to Influenza virus.

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**Fig. Detection of virus nucleic protein in infected cells using microscopic imaging**

The state of virus penetration in infected cells was observed with a confocal laser scanning microscope using FITC-labeled Abs of nucleocapsid protein of influenza virus. Nuclei were stained with DAPI. Data are expressed as no-treatment, virus control and Hochu-akkito treatment at the adsorption stage and post-entry.

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**65 Antiviral Activity of a Phenolic Dibenzylsulfide Against New World Clade B Arenavirus Infections**

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Junin and several other Clade B New World arenaviruses cause human disease ranging from mild febrile illness to fatal viral hemorrhagic fever (HF). Ribavirin is the only licensed antiviral drug available to treat arenaviral HF; however, there is limited data to support the use of ribavirin to treat serious infections with New World HF viruses and therapy is associated with toxicity. Screening of the Chemtura library identified several compounds with activity against Tacaribe virus (TCRV), a Clade B New World arenaviruses closely related to Junin virus (JUNV). Of these compounds, D746, a phenolic dibenzylsulfide, was further pursued. D746 inhibition of TCRV-induced cell death was confirmed to be in the micromolar range by virus yield reduction assays using both TCRV and the Candid 1 vaccine strain of JUNV. In contrast, no activity was found when the compound was evaluated against the Pichinde arenavirus (Clade A). Based on its activity in vitro, D746 was evaluated in the AG129 mouse TCRV infection model. When initiating treatment 2 h prior to virus challenge, a one week dosing regimen of 50-200 mg/kg of D746 divided into two daily doses resulted in significant protection (70-100% survival) compared to mice treated with placebo (~20% survival). Despite the remarkable effect of the compound on protecting mice from mortality, there was no impact on serum or tissue virus titers. This result was surprising considering the activity observed in vitro. Additional experiments are underway to gain insights into the possible mechanism(s) of action.
and post-exposure treatment efficacy. Supported by contracts N01-AI-30048, N01-AI-30063, HHSN272201100019I, and HHSN272201000039I, Virology Branch, NIAID, NIH.

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Antiviral activity of Ladania067 an extract from *Ribes nigrum* against influenza- and rhinovirus

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Influenza- and rhinovirus are the most frequent courses of acute respiratory tract infections. Influenza virus is found in the upper and lower respiratory tract and can lead to severe clinical outcomes. In contrast, rhinovirus, the causative agent of the common cold is predominantly present in the upper respiratory tract. For both pathogens new antiviral agents are needed in order for a better control infections. In this regard, we were able to show that a plant extract from *Ribes nigrum* named Ladania067 exhibits antiviral activity against influenza- and rhinoviruses *in vitro* and in a mouse model. We could show that the IC₅₀ values for prophylactic-treatment against pandemic influenza A (H1N1) 2009 was 50ng/ml, while IC₅₀ values against rhinovirus was roughly 10-fold higher. Antiviral treatment starting at different time-points after infection was more potent against rhinovirus compared to influenza virus. Mechanistically, from our experiments we conclude that the extract mainly inhibits virus entry but it also exhibits additional intracellular functions that may interfere with viral replication. In addition to investigations using the full extract from *Ribes nigrum* leaves, we performed experiments to scrutinize the antiviral potential of HPLC fractions derived from the Ladania067 extract. Here, we could show that various fractions demonstrated strong antiviral potential against either influenza- or rhinovirus. A reduction of virus titer was also observed after Ladania067 treatment in lungs of influenza- or rhinovirus infected mice. The data demonstrates that Ladania067 is effective against influenza- and rhinovirus, most probably due to broad specificity, which is commonly found in plant extracts. Therefore, we conclude that Ladania067 might be an effective antiviral with prophylactic and therapeutic potential against influenza- and rhinovirus, the main etiological agents causing acute respiratory tract infections.

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The Combination of 4'-Ethynyl-2-Fluoro-2'-Deoxyadenosine with Rilpivirine Shows Synergistic Anti-HIV-1 Activity *in vitro*

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4'-Ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) is a potent HIV-1 inhibitor that blocks the translocation of HIV-1 reverse transcriptase (RT). Drug combination studies of EFdA with FDA approved drugs are critical to determine optimal inhibitor combinations prior to initiation of clinical trials. We performed drug combination susceptibility studies both in viral and enzymatic assays and evaluated them using MacSynergy II and CalcuSyn softwares.
Tenofovir and EFdA, which are both adenosine analogs, were expected to compete with for incorporation at the same template positions. However, this combination did not exhibit antagonism, but moderate additivity. In contrast, EFdA appeared to compete with lamivudine (3TC) or emtricitabine (FTC), even though they are incorporated at different template sites. The moderate antagonism between EFdA, FTC and 3TC is likely due to competition for phosphorylation by the identical cellular activating enzyme (2′-deoxycytidine kinase, dCK).

Interestingly, EFdA acted synergistically with rilpivirine (RPV), a diarylpyrimidine (DAPY) derivative that belongs to the nonnucleoside RT inhibitor (NNRTI) class of antivirals, when tested in cell-based and enzymatic assays. This study demonstrates that EFdA can be used very effectively in combinations with other drugs that are used in HIV-1 therapy and provides information that is potentially useful for the design of combination regimens for initial and salvage therapy.

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Identification of small molecules that inhibit Tat-mediated HIV-1 replication by in silico screening targeting human cyclin T1

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HIV-1 transcription is the only step for amplifying viral genomes and is essential for viral replication. However, until now, drugs that inhibit this step have not been used for treatment of HIV-1-infected patients. Cyclin T1 (CycT1) interacts with HIV-1 Tat and TAR RNA, and their complex activates viral transcription through the hyperphosphorylation of RNA polymerase II. The crystallographic structure of CycT1-Tat-TAR RNA complex has recently been elucidated, which provides a target site for the structure-based drug design to develop novel HIV-1 transcription inhibitors. In this study, we conducted in silico screening of compounds targeting the Tat-TAR RNA binding site of CycT1 using the molecular docking simulation software MOE (Chemical Computing Group Inc.). Among three million compounds, we selected 124 compounds with high docking score and examined for their anti-HIV-1 activity in vitro, resulting in the discovery of two selective inhibitors of HIV-1 replication (Compound 1 and Compound 2). When we searched their derivatives, one compound was identified as a more potent inhibitor of HIV-1 (Compound 3). Compound 3 was found to inhibit the Tat-induced HIV-1 LTR-directed transactivation of a reporter gene in T-cell lines. Furthermore, according to the docking scores and anti-HIV-1 activity of the three compounds, the docking pose of Compound 3 with CycT1 was predicted. In this model, Compound 3 interacted with V199, L203, F241, K253, and R259 of CycT1 and masked the surface of Q172 and F176. Since Q172, F176, and R259 are known to interact with HIV-1 Tat, these results suggested that Compound 3 blocked the interaction between CycT1 and Tat and inhibited the HIV-1 replication. Further analysis of Compound 3 for its mechanism of action is currently in progress.

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Towards HIV Eradication: Excision of HIV-1 Proviral DNA using LTR-Specific Recombinase

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Background HIV-1 integrates into the host chromosome and persists as a provirus flanked by long terminal repeats (LTR). To date, treatment regimens primarily target the virus enzymes or virus entry, but not the integrated provirus. Therefore, HAART requires lifelong treatment which is frequently accompanied by the
occurrence of substantial side effects and/or the development of drug-resistant viruses. Previously, we engineered a LTR-specific recombinase (Tre-recombinase) that effectively excises integrated HIV-1 proviral DNA from infected human cell cultures, suggesting that customized enzymes might someday help to eradicate HIV-1 from the body. Therefore, we here analyzed the potential of Tre-recombinase to reverse HIV-1 infection in vivo. Methods We constructed an advanced lentiviral self-inactivating (SIN) vector that expresses Tre-recombinase conditionally in HIV-infected cells and monitored Tre functionality and potential Tre-related cytopathic effects over time in tissue cultures. Moreover, the effect of Tre activity on HIV-1 infection was investigated in humanized mice. Results It is shown that Tre-recombinase is efficiently delivered into cells and accurately excises HIV-1 proviral DNA from chromosomal integration sites. Apparently, prolonged overexpression of Tre-recombinase does not induce undesired cytopathic effects in the transduced cells. Finally, we demonstrate pronounced antiviral activity of Tre-recombinase in HIV-1 infected Rag2-/-gc-/- mice, which were either engrafted with Tre-transduced human CD4+ T cells or with Tre-transduced human CD34+ hematopoietic stem cells (HSC). Conclusions The presented data suggest that Tre-recombinase may be a valuable component of future antiretroviral therapies of the post HAART era that aim at virus eradication, thereby providing a cure for AIDS.

70 Enhancement of HIV therapeutic vaccination of Th17 GALT cell lines with LAMP, Interleukin-22 and Ganeden BC30 to control HIV infection.

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Background HIV therapeutic vaccination remains an elusive milestone. Improving the efficacy of ARV’s may be effectively addressed by preventing loss of Th17 cells. Strategy aimed at combining enhancement of innate immunity, increase of antigen presentation to therapeutic vaccine and improvement of GALT immunity may be a viable way to complement or potentially replace existing ARV therapies. Interleukin-22 (IL-22) is a cytokine produced by immune cells (TH 17 and NK cells) and acts on tissue cells playing pivotal role in preventing and treating infections including HIV. IL-22 and BC 30 probiotic may be synergistic and enhance therapeutic vaccination responses when antigen presentation is facilitated by lysosomal associated membrane protein-1 (LAMP-1). LAMP mediated immunization drives prolong immunological memory to Gag epitopes in experimental models. Methods Mucosal cell cultures were co-cultured with exposed to IL-22 and supernatants of BC probiotic bacteria in the presence of peripheral blood mononuclear cells (PBMCs) from HIV infected patients to measure anti microbial factors and inhibition of HIV infectivity. The ability of LAMP-targeted antigen construct to enhance MHC II presentation was measured using autologous antigen presenting cells (APC) to induce proliferation of CD4T cells. Results Independently produced in vitro and in vivo studies modeling the efficacy of IL-22 and BC30 probiotic and LAMP show effect on viral replication including viral latent reservoirs.Hidden formatting deleted. Delete this text! yes>> Intradermal delivery of the LAMP/ HIV-1 gag DNA vaccine elicits enhanced Ab production and amplify anti-Gag CD4+, CD8+ T and B cells responses LAMP DNA vaccines target antigenic sequences directly to MHC-II compartment resulting in direct loading of the MHC-II molecule during the lysosome maturation process. BC30 probiotic may contribute to restoration of CD4+ T cells responses, improve GALT immunity, decrease inflammation and microbial translocation.
Standardized cell-based assays to evidence antiviral effects of new compounds against arbovirus

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Arboviruses such as Chikungunya virus or dengue virus (CHIKV and DENV, respectively) cause severe infections leading to arthralgia, chronic arthritis but also encephalitis or viral hemorrhagic fever that can be fatal in humans. Along with mosquito control and with the development of effective vaccines, a research for new antiviral compounds against arboviruses is also a way of fighting these epidemic diseases. Cell-based assays developed so far measured viral replication by counting plaque-forming units (pfu). This method is time consuming and not really standardized. As a consequence, we developed a screening microassay (today, 96-well microplate coupling the culture of HEK293 cells with a specific EIA designed to quantify viral replication. Cytotoxicity is quantified in parallel using the same cells but not exposed to the virus and the MTS/PMS colorimetric method. This problematic is especially interesting that few effective antivirals are today described against arboviruses. This microassay was validated using 3 reference compounds, chloroquine and two nucleoside derivates (i.e. ribavirin and 6-azauridine). In our cell model, only chloroquine demonstrated slight antiviral effects (selectivity index: 1 – 4) for all viruses tested today i.e. CHIKV Ross river virus (RRV) and the serologically distinct DENV serotypes. Nucleoside derivates i.e. Ribavirin and 6-azauridine are only efficient against DENV (Selectivity index: 15 - 30). These low antiviral efficiencies justify the screening of new compounds against different arboviruses using standardized cell-based microassays.

Impact of Short Term Anti-Retroviral Therapy (START) on some Fibrinolytic Markers in some HIV Infected Adults: Preliminary Findings from the START study.

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Background: Derangement in fibrinolytic markers can result in thrombosis and cardiovascular problems and anti-retroviral therapy (ART) has been reported to affect the levels of these markers. How long the patients will be exposed to these ART before the effect of the drugs on the fibrinolytic markers become noticeable is not well known. This START study was aimed at bridging this knowledge gap. Methods: 20 HIV subjects on ART and 20 controls (non- ART) were progressively monitored for three months. CD4 T cell count was determined using the Partec Flow cytometry counter while D-dimer, t-PA and PAI-1 parameters were determined using ELISA kits from TECHNOCLONE, Austria. Results: CD4 cell count increased from 192m/ml at baseline to 323 µl/ml at month 3 among patients on antiretroviral therapy. D-Dimer values decreased from 301.0µl/ml at baseline to 172.0 µl/ml at month 2 and increased significantly to 226.0 µl/ml at the end of the third month. The median baseline value of PA1-1 at the beginning of therapy was 14.0mg/ml, which increased progressively to 18.2mg/ml at the end of the third month. The baseline value of t-PA at the beginning of therapy was 5.15mg/ml. This value progressively declined to 1.10mg/ml at the end of the first month and increased minimally to 1.45mg/ml and 1.5mg/ml at the end of the 2nd and 3rd month respectively. D-dimer was positively and significantly correlated with CD4 cell counts in both ARV and non-ARV patients. (r = -0.304, p<0.01 vs. r = -0.477; p<0.001). t-PA was negatively and significantly
correlated with CD4 T lymphocytes in only those undergoing the antiretroviral therapy (r = -0.294, p<0.01).

**Conclusion:** A progressive increase in PAI-1 value and steady decline in t-PA values within three months of commencement of antiretroviral therapy is capable of predisposing the patients to thrombotic disorders earlier than is expected. Pre-thrombotic assessment during therapy is hereby advocated.

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**Diagnosis of influenza viruses by peptide nucleic acid-modified with a novel intercalator**


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Influenza A virus is a member of the Orthomyxoviridae, which is a family of enveloped viruses with segmented, single-stranded, negative-sense RNA genomes. The genome of influenza A virus consists of eight single-stranded, negative-sense RNA segments (size from 890 to 2341 bases) that form ribonucleoprotein complexes (RNPs) together with the viral RNA (vRNA)-dependent RNA polymerase complex and many nucleoprotein (NP) molecules. We identified a highly conserved 15 base sequences on the NS genome that is common and specific to influenza A/H1N1 strains by a software CONSERV. We employed peptide nucleic acid (PNA), a DNA/RNA analogue, in which the phosphate backbone has been replaced by a neutral amide backbone for the diagnosis of influenza A(H1N1) virus. The binding properties of PNA to the target viral RNA were evaluated by monitoring the inhibitory effect on reverse transcription (RT) of MuLV reverse transcriptase in RT-polymerase chain reaction (RT-PCR). As a result, the anti-NP PNA recognized the virus genome within the RNPs in a sequence specific manner. Interestingly, PNA-SS which was modified with our newly synthesized intercalator (SS) showed nearly 10-fold higher inhibitory effect. PNA-SS efficiently captured the RNPs of influenza A(H1N1) on a plate. The RNPs were easily visualized by the naked eyes using enzyme-linked immunosorbent assay and the threshold for the detection of influenza A(H1N1) virus was 6×10⁴ pfu/ml. This technology can be useful for the diagnosis of influenza viruses without requiring the use of expensive equipment or electricity. Meanwhile, we also developed a real-time quantitative RT-PCR system for the detection of oseltamivir-resistant influenza A(H1N1) viruses carrying a neuraminidase (NA) gene with an H274Y amino acid substitution using anti-NA PNA as a single nucleotide polymorphism(SNP) probes. This technology is applicable for the detection of drug resistance arises from mutations in the viral genome.

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**Chronic treatment with Azidothymidine (AZT) alters cytoskeletal proteins responsible for cardiac function**


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Metabolic complications resulting in cardiomyopathy are associated with long-term treatment of nucleoside reverse transcriptase inhibitors (NRTIs). While mitochondrial toxicity is thought to be a major underlying cause, the molecular mechanism(s) are still ambiguous. In the current investigation we are interested in identifying molecular targets and mechanisms leading to cardiac toxicity. We chronically exposed adult male rats to AZT, provided in
drinking water for a period of six months. At the end of the study, rats were subjected to an echocardiogram. We observed mild changes in the ECHO data suggesting impaired cardiac function in AZT treated rats. The respiratory control rates and electron transport chain capacity of mitochondria isolated from these rat hearts were studied polarographically using a high resolution respirometer and we did not observe any significant changes. Also there was no change in the mitochondrial DNA copy numbers in treated rats. Interestingly, microarray of total mRNA from these heart samples presented with dramatic alterations in expression of mRNA in rats treated with AZT. Among these mRNAs, cytoskeletal protein mRNA alterations were evident in a number of pathways. Cytoskeletal/contractile proteins such as actin, coflin, profilin, myosin II, myosin light chain and myosin heavy chain which contribute to contractile functions were significantly altered. A similar alteration in mRNA expression was also noted in microarrays from liver and skeletal muscle mRNA, however, the extent of mRNA expression differences were less than those observed in heart tissue. Taken together, the data suggest that cardiac toxicity in a chronic treatment regimen of male rats involving AZT may not be associated with mitochondrial toxicity.

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Nucleozin elicits rapid and target-specific aggregation of influenza A nucleoprotein (NP)
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The recent emergence of human-infecting influenza A viruses of animal origin has reminded us of the possibility of an influenza pandemic with devastating outcomes comparable to that of the 1918 Spanish flu (claiming more than 20 million lives). Available anti-influenza drugs such as M2 ion channel inhibitor amantadine and neuraminidase inhibitor oseltamivir are becoming less effective due to rapid emergence of drug-resistance viruses. We have recently identified influenza A nucleoprotein (NP) as a novel druggable target for anti-influenza drug development and simultaneously presented a NP-interacting small molecule compound nucleozin as a potent antiviral agent (Kao et al., Nature Biotechnology, 2010). Our findings were quickly validated by two subsequent independent studies published in PNAS (Su et al., 2010; Gerritz et al., 2011), illustrating the impact and significance of identifying influenza A NP as a new antiviral target. We further demonstrate that nucleozin would elicit rapid aggregation of influenza A NP in a target-specific and time-dependent manner in in vitro cell-free assays with purified recombinant NP and at cellular level with influenza A virus infected or NP-expressing plasmid transfected cells. The aggregation of NP apparently halts the cellular trafficking of NP and thus abolishes the biological activities of this crucial component of the virus. Our recent collaborative work with Professor Digard’s laboratory demonstrates that the addition of nucleozin to virus-infected cells stops cellular trafficking of newly synthesized viral ribonucleoproteins (RNP). This is the first time a small molecule compound is shown to halt the cellular movement of the assembled RNP, which is thousands of times larger than the nucleozin. Understanding the underlying mechanism of nucleozin-induced NP/RNP aggregation would help us elucidate the mode of action of nucleozin and gain more insight in compound-induced specific aggregation of macromolecules, an area that has yet to be explored.

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Inhibition of Influenza Virus Entry by Epigallocatechin Gallate, the Green Tea Flavonoid
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Epigallocatechin gallate (EGCG), the main polyphenol component in green tea, has been proposed to have antiviral activity against several enveloped viruses including influenza virus as well as human immunodeficiency virus, herpes simplex virus and hepatitis C virus. However, its mode of action against influenza virus has not yet been elucidated sufficiently. In this study, in vitro antiviral activity of EGCG and its derivatives was investigated by cytotoxic effect reduction assay using fluorescein diacetate. The results showed that EGCG has very low cytotoxicity (CC₅₀ >100 mM) and is active not only against influenza A viruses [H1N1: A/Taiwan/1/86, A/Puerto Rico/8/34 (PR8) and A/Brisbane/59/2997 H3N2: A/Hong Kong/8/68] with EC₅₀ values 10−20 mM but also against influenza B virus (B/Panama/45/90) with EC₅₀ approximately 30 mM. In a time-of-addition experiment, inhibition of PR8 virus plaque formation by EGCG was detected when the compound was co-treated with virus (EC₅₀, about 10 mM) rather than when it was treated before or after virus infection. It is worth noting that this antiviral activity was significantly improved by preincubation of EGCG with virus for 1 h (EC₅₀, <0.08 mM). It means that EGCG could inhibit influenza virus entry by functional modification of hemagglutinin (HA) required for binding to the receptor or for membrane penetration, but not by inactivation of cell surface receptors. Fluorescence microscopy using antibodies specific for viral nucleoprotein or whole influenza A (H1N1) virus particles showed that entry of PR8 virus into MDCK cells is suppressed by EGCG in a dose-dependent manner. The finding that EGCG acts as a potential influenza entry blocker provides a perspective for further understanding on the broad spectrum antiviral activity and an approach for improving its antiviral efficacy by chemical modification. **Acknowledgement:** This work was supported by Transgovernmental Enterprise for Pandemic Influenza in Korea, TEPIK (Grant A103001).

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**IDENTIFICATION OF NEW AND NOVEL TYPES OF INHIBITORS AGAINST HIV-1**

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Even though the HAART therapy against HIV/AIDS has been successful for controlling the disease so far, however, the emergence of a case of AIDS in New York recently that is resistant to 17 of the 20 marketed drugs is a reminder that the development of new anti-HIV/AIDS drug targets and drugs or intervention is urgently needed to overcome the current drug resistance problems. One of such possible targets that could solve the problem has been the HIV Nucleocapsid (NC) protein which is derived from the HIV Gag polyprotein and is involved in almost all of the viral life cycle, especially in the packaging of viral RNA genome into virus particles, as the protein is being known as "mutation non-permissive" in its nature. Yet, the inhibitors against the protein have been very limited a certain type of chemicals due to the lack of an efficient HTS screening technology. By developing and exploiting a novel and innovative cell-based assay for the first time (Journal of Virology 81(11) 6151~p6155, 2007) which probes NC protein function within living cells, we have screened about one hundred thousand chemical libraries and isolated a number of small molecule chemical inhibitors against the HIV NC protein. These new anti-HIV molecules comprise of novel types of chemical inhibitors targeting the HIV NC protein, which show potent anti-HIV viral activity with a novel mechanism of action as well as low cellular toxicity. The identification of these novel anti-HIV inhibitors may open up a new aspect of developing of a new anti-HIV drug and dealing with the resistance problems of current HIV/AIDS drugs.
IC_{50}s of neuraminidase inhibitors to influenza viruses are highly variable dependent on experimental conditions
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A Novel Strategy for Efficient Production of Anti-V3 Human ScFvs against HIV-1 Clade C
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Background: Production of human monoclonal antibodies that shows broadly neutralizing activity is needed for the prevention of HIV-1. Here we devised a novel approach and produced 11 different human scFvs against the V3 region of HIV-1 envelope. Method: The peripheral blood mononuclear cells (PBMCs) were isolated from an HIV-1 clade C infected drug naïve Indian patient whose plasma exhibited neutralizing antibodies against a panel of viruses (5 Clade C & 3 Clade B). PBMCs were EBV transformed in 96 well plate and then expanded into 24 well stage followed by 6 well and finally to flask stage. The transformed cells (wells) were screened against HIV-1 consensus C V3 peptide at each stage and only the positive cells (wells) were carried onto the next stage. Total RNA from these enriched positive antibody secreting cells at the flask stage was isolated and cDNA was synthesized. VH and VL genes were amplified using specific primers and scFvs were constructed, cloned into phagemid vector and transformed into E.coli TG1. Results: By EBV transformation and preselection of V3 specific clones we successfully constructed a small phage library of 7000 clones. A total of 400 ng of digested scFv DNA was ligated into 1 µg of phagemid vector. One round of bio panning was done against HIV-1 consensus C and B V3 peptides. Randomly, 40 clones were selected and checked for their binding. Out of 40 clones, 13 showed positivity in phage ELISA. DNA fingerprinting analysis using BstN I followed by sequencing showed that 11 out of 13 clones were distinct. Expression of positive clones was tested by SDS-PAGE and Western blot. Specificity of these positive clones was checked by ELISA against the V3 peptides. All the 11 anti-V3 scFvs showed cross-reactivity against both the V3 peptides and did not show any reactivity against other unrelated peptides. Conclusion: This is the first study to generate anti-V3 scFvs against HIV-1 Clade C. Further assessment of the neutralization efficiency of these scFvs would reveal their potential for passive immunotherapy.

Roles of NS5B amino acids 15, 223, and 321 in HCV Replicon Replication Using Mutagenesis and Crystallization of the NS5B Polymerase
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Resistance to the HCV inhibitors 2′-F-2′-C-methylguanosine monophosphate nucleotides, PSI-352938 and PSI-353661, is associated with a combination of amino acid changes in NS5B. In particular, the S15G, C223H, and V321I mutations were identified through selection studies in genotype (GT) 2a JFH-1 derived replicon cells. In order to better understand the roles of these amino acids in HCV replicon replication, mutagenesis studies were performed on each of these amino acids. Residues 223 and 321 are both located within the palm domain close to the catalytic D220 and D318 residues, but the side chains of the amino acids are directed away from the active site. To
determine whether size and polarity at position 223 could affect replication, Ser, Gln, Trp, Asp, or Arg were
evaluated. V321 was changed to Ala, Lys, or Phe to examine the impact of size variation. Residue 15 is located on
the surface of the finger domain and may interact with the incoming RNA template. To determine the effect of
polarity, Ser was changed to Glu, Asp, Lys, Arg, or Asn. Results from the C223 studies showed that this position
 favored a neutral residue over a polar one and that large and bulky residues reduced the replication capacity of
HCV replicons. In contrast, position 321 preferred larger residues over the smaller Ala and that resistance to PSI-
352938 appeared to be partially dependent on the size of the amino acid. Although residue 15 is not located within
the active site, mutagenesis at this position impacted replication capacity of the HCV replicons. Data from both GT
1b and 2a replicon mutants showed that an acidic residue at position 15 severely impaired replication. Finally, the
JFH-1 NS5B polymerase containing all three resistant mutations (S15G/C223H/V321I) was crystallized. Remarkably,
the triple mutant showed a more "open" structure compared to the wild type polymerase. Together these data
indicate that residues 15, 223 and 321 regulated the replication of HCV replicons and that the three resistant
mutations could modulate the conformation of the polymerase, which may result in modifying the interaction with
the RNA and/or nucleotide substrates during RNA synthesis.

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Sublingual administration of Lactobacillus rhamnosus facilitates protection against influenza virus infection in
mice
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Infection with influenza viruses is a significant cause of morbidity and mortality all over the world. In previous
studies, Lactobacilli were found to be effective in the prevention of influenza virus infection in mice. Recently, it
has been demonstrated that sublingual (s.l.) mucosa is an efficient site for the induction of broad-spectrum of
immune responses. In this study, we examined the protective efficacy of s.l. administration of live Lactobacillus
rhamnosus (L. rhamnosus) against challenge with influenza virus in mice. Moreover, we performed immunologic
assays to understand the underlying mechanism. Mice were administrated with L. rhamnosus (10^6–10^8 cfu/mouse)
by s.l. for 10 days. Subsequently, mice were inoculated intranasally with influenza virus A/NWS/33 (H1N1) strain,
observed daily for 14 days. For immunologic analysis, we analyzed viral load, IgA levels, NK cell activity and
cytokine profiles in the lung of mice. In a mouse challenge study, s.l. administration of L. rhamnosus reduced
mortality in a dose-dependent manner. In immunologic analysis, significant increase of IgA production and NK cell
activity in the lung was observed and accompanied with remarkable decrease of lung virus titers after s.l.
administration. Moreover, the levels of IL-12 in the lung were significantly increased, but the levels of TNF-α and
IL-6 were conversely decreased. In this study, s.l. administration of L. rhamnosus can protect influenza virus
infection in mice and increase IL-12 production and NK cell activity in the lung. Significant increase of IL-12 might
augment NK cell activity and be related to further decrease of viral load in the lung of mice, as previously reported.
Since fatal outcome of influenza is associated with cytokine imbalance including hypercytokinemia, the decrease in
TNF-α and IL-6 levels may also contribute to protection against influenza virus. Therefore, s.l. administration of L.
rhamnosus determined in this study has potential of preventive measures against seasonal influenza as well as the
next pandemic influenza.
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Structural insights into RNA 2'-O methylation by the flavivirus NS5 protein

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@font-face { font-family: "Calibri"; @font-face { font-family: "SimSun"; } p.MsoNormal, li.MsoNormal, div.MsoNormal { margin: 0cm 0cm 0.0001pt; font-size: 12pt; font-family: "Times New Roman"; } div.Section1 { page: Section1; } The N-terminal domain of the flavivirus NS5 protein sequentially methylates the viral genomic RNA as follows: G5'\textsubscript{ppp}S'\textsubscript{A}→\textsuperscript{7me}G5'\textsubscript{ppp}S'\textsubscript{A}→\textsuperscript{7me}G5'\textsubscript{ppp}S'\textsubscript{A}\textsubscript{2'-O-me}. However, a molecular view of how methylation is performed is still elusive. We present a crystal structure at a resolution of 2.5 Å that contains two ternary complexes between the Dengue virus methyltransferase (MTase), a N7-methylated RNA substrate and S-adenosyl-L-homocysteine. Only the 5' end of the capped RNA is well ordered in the basic binding groove and the two RNA molecules adopt extended but slightly different conformations. This illustrates the ability of the flavivirus MTase to establish alternative contacts with short capped-RNA. The conformation adopted by the capped RNA is consistent with a 2'-O methylation mode: the 2'-OH of its adenine ribose lies in close proximity to the sulphur atom of SAH and adjacent to the side chain of K180 from the conserved KDKE catalytic motif. Based on these structural observations and mutagenesis data, we propose a catalytic mechanism for 2'O methylation by the flavivirus NS5 protein and discuss implications for antiviral drug discovery.

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"Resistance Analysis and Characterization of a Thiazole Analogue, BP008, as a potent Hepatitis C Virus NS5A Inhibitor"

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Hepatitis C virus (HCV) is a global health problem, affecting approximately 3% of the world's population. The standard treatment for HCV infection is often poorly tolerated and ineffective. Therefore, the development of novel or more effective treatment strategies to treat chronic HCV infection is urgently needed. In this report, BP008, a potent small-molecule inhibitor of HCV replication was developed from a class of compounds with thiazol core structure by means of utilizing a cell-based HCV replicon system. The compound reduced the reporter expression of the HCV1b replicon with an EC\textsubscript{50} and selective index value of 4.1 ± 0.7 nM and >12,195, respectively. Sequencing analyses of several individual clones derived from BP008-resistant RNAs purified from cells harboring HCV1b replicon revealed that amino acid substitutions mainly within the N-terminal region (domain I) of NS5A were associated with decreased inhibitor susceptibility. Q24L, P58S and Y93H are the key substitutions for resistance selection; F149L and V153M play the compensatory role in the replication and drug resistance processes. Moreover, BP008 displayed synergistic effects with interferon alpha (IFN-\(\alpha\)), NS3 protease inhibitor, and NS5B polymerase inhibitor, as well as good oral bioavailability in SD rats and favorable exposure in rat liver. In summary, our results presented an effective small-molecule inhibitor, BP008, potentially targeting at HCV NS5A. BP008 can be considered as part of a more effective therapeutic strategy for HCV in the future.

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Structure-based inhibition of norovirus RNA-dependent RNA-polymerases

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Caliciviridae are RNA viruses with a single-stranded positively-oriented polyadenylated genome, responsible for a broad spectrum of diseases such as acute gastroenteritis in humans. Recently, analyses on the structures and functions of the RNA-dependent RNA-polymerase (RdRp) from several Caliciviruses have been reported. The RdRp domains have been shown to share the typical "right hand" overall structure, with differences localized in the C-terminus in the palm domain. Moreover, the structure of hNV bound to template and primer RNA provided a high resolution description of the protein during RNA elongation. Starting from the crystal structures of human norovirus RdRp (hNV), we performed an in silico docking search to identify synthetic compounds with predicted high affinity for the enzyme active site. The best ranked candidates were tested in vitro on murine norovirus (MNV) and hNV RdRp to assay their inhibition of RNA polymerization. The results of such combined computational and experimental screening approach led to the identification of two high-potency inhibitors: Suramin and NF023, both symmetric divalent molecules hosting two naphthalene-trisulfonic acid heads. We present the crystal structures of MNV RdRp bound to the two identified inhibitors. Both inhibitory molecules occupy the same RdRp site, between the fingers and thumb domains, with one inhibitor head close to residue 42 and to the protein active site. To further validate the structural results, we mutated to Ala Trp42 in MNV RdRp, and the corresponding residue (Tyr42) in hNV RdRp, showing decrease of inhibitory potency of the two compounds for both the mutated proteins.

Mechanism of Ivermectin-mediated flaviviral helicase inhibition

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Infections with yellow fever virus (YFV) and other emerging and re-emerging pathogenic flaviviruses [such as dengue (DENV), West Nile (WNV), and Japanese encephalitis (JEV) viruses] pose a serious global public health problem. Potent and safe antivirals are urgently needed. Based on 3D structures of WNV helicase domain, we explored a novel yet unexploited protein site for ligand binding, screening in silico thousands of low molecular weight compounds. Among the compounds with high predicted affinity for the new site, Ivermectin has been proved to be a highly potent inhibitor of YFV replication (EC50 values in the sub-nM range) and to a lesser extent of DENV, JEV and tick-borne encephalitis viruses (EC50 values in the sub-microM range). Time-of-drug addition studies suggested that Ivermectin inhibits YFV and DENV replication at a time point matching the onset of intracellular viral RNA synthesis, as expected for a molecule that targets the helicase. Using in vitro enzymatic assays, employing recombinant NS3 helicases of different flaviviruses (YFV, DENV and WNV), we confirmed that Ivermectin inhibits the dsRNA unwinding activity in all the tested helicases (IC50 values in the sub-microM range). Kinetic studies showed that Ivermectin is an uncompetitive inhibitor respect to dsRNA in all the flaviviral enzymes investigated, indicating that the compound is able to bind to the protein only when RNA is present. In the absence of crystal structures, we simulated a number of possible conformations of the ligand inside the ssRNA access site. The results allowed the identification of two conserved amino acids (T408 and D409 in DENV helicase) whose
mutation was predicted to impair Ivermectin binding. Accordingly, DENV, WNV and YFV helicases double mutants (T>C and D>E) were generated. The mutated proteins conserved helicase activity but were not inhibited by Ivermectin (up to 5 microM). This result provides strong evidence that the two selected amino acids (or one of the two) interact with the inhibitor, confirming the binding of Ivermectin to the ssRNA access site.

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CHEMOKINE RECEPTORS CXCR4 AND CCR5 IN HIV/HCV COINFECTED PATIENTS

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Aim of study: to evaluate expression of chemokine receptor CXCR4 and CCR5 on T lymphocytes in patients with HIV/HCV coinfection. Material and methods. Markers of activation and suppression of immunity response (IR) (HLA-DR, CD25, B-cells, T-cells and its' subpopulations and chemokine receptors CXCR4 and CCR5) have been studied in 3 groups of patients: the 1st group – 51 patients with coinfection HIV/HCV, the 2nd group – 23 patients with HIV-infection, the 3rd group – 10 patients with HCV-infection. Control group consisted of 16 healthy adult persons. Monoclonal antibodies (Becton Dickinson, USA) have been used. Results. In the 1st group the expression of CXCR4 by blood lymphocytes was decreased in comparison with control and at the same time the expression of CCR5 by blood lymphocytes was increased in comparison with HVC-infected group of patients. The expression of CXCR4 in the 1st group has been positively correlated (Sperman correlation, p<0,05) with markers of IR suppression (CD25/CD4, CD3+CD16+CD56+) and CD3+CD19+, CD3+CD4+. Negative correlation of CXCR4 was established with CD3+CD8+ and CD3–CD16+CD56+. The expression of CCR5 in the 1st group has been positively correlated with markers of IR activation (HLA-DR, CD3+HLA+, CD3+CD8+HLA+) and CD3+CD8+, CD3–CD16+CD56+. Negative correlation of CCR5 was established with markers of suppression of IR (CD25/CD4, CD3+CD16+CD56+) and CD3+CD4+, CD3+CD19. Conclusion. Expression of CCR5 by blood lymphocytes was increased in HIV/HCV coinfection in comparison with HVC-infected group of patients. Increased expression of CXCR5 has been associated with activation of immune system and Th1 immune response. Results of study assume stronger Th1 immune response in HIV/HCV coinfected patients in comparison with HVC-infected patients.

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Effect of 4'- and 2-NRTI substitutions on the inhibition mechanism of HIV Reverse Transcriptase and toxicity
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Recently, we showed that 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) inhibits HIV-1 with greater potency than other known inhibitors by blocking the translocation of HIV-1 Reverse Transcriptase (RT) (Michailidis et al., 2009). In order to elucidate the role of 4'- and 2-groups we studied the effect of substitutions at these positions into the biochemical mechanism of HIV-1 RT inhibition and toxicity. These compounds are 4'Me-dATP, 4'Me-dTTP, 4'Et-dTTP, 4'Et-dATP, ENdA-TP and D-carba-TTP. Our in vitro studies demonstrate that ENdA-TP is the most potent inhibitor on both RNA/DNA and DNA/DNA template-primer, followed closely by Efda-TP. Although all of these inhibitors have a chemically active 3'OHH group, they generally act as obligate chain terminators by inhibiting DNA polymerization primarily at the point of incorporation. However, depending on the template sequence, they may

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also inhibit RT to a lesser extent as delayed chain terminators. Fe\(^{2+}\)-mediated footprinting assays showed that their ability to act as obligate chain terminators correlates with their ability to prevent RT translocation. To assess possible mitochondrial toxicity of these compounds we tested their ability to inhibit human mitochondrial DNA polymerase g (pol g). We found that ENdA-TP and 4'M-dTTP were the most potent inhibitors of pol g (IC\(_{50}\) of ~50 \(\mu\)M). We also identified a correlation of the cytotoxicity of these nucleoside analogs with the morphology and number of mitochondria, and the depletion of mitochondrial DNA.

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Sustained Activity of 4'-Ethynyl Nucleosides to Variants with M184V Mutation in HIV-1 Reverse Transcriptase
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[Background] 4'-Ethynyl (E)-2-fluoro-2'-deoxyadenosine (EFdA) and 4'-E-2'-deoxyribofuranosyl-2,6-diaminopurine (EdDAP), members of NRTIs, exert potent activity against multi-drug-resistant HIV-1 and HIV-2 and have favorable safety features with \(qd\) dosing possibility. Previous in vitro resistance selection with 4'-E-2'-deoxyadenosine (EdA), a lead compound for EFdA and EdDAP, revealed that I142V, T165R and M184V were associated with the resistance. HIV-1\(_\text{I142V/T165R/M184V}\) showed 39-fold resistance to EdA and cross-resistance to EdF (22-fold). However, the mechanism(s) of resistance to EFdA and EdDAP remains to be elucidated. [Findings] We selected resistant HIV-1 and HIV-2 to EFdA and EdDAP with the dose-escalating method. During selection, M184I and M184V emerged with a few substitutions combined. In the drug susceptibility assay of recombinant HIV-1 clones, M184I/V conferred ~10-fold resistance to EFdA, but <3-fold to EdDAP. Other substitutions conferred moderate resistance, but those combined with M184I or M184V conferred substantial resistance. Our further examinations revealed that M184V is a primary substitution for EFdA and EdDAP resistance, in which the exclusion mechanism was involved; yet EC\(_{50}\) values of EFdA and EdDAP against such resistant variants remained comparatively low to that of AZT. To address what factor(s) is(are) involved in such sustained activity, we further examined the stability of EFdA and EdDAP against adenosine deaminase (ADA). EFdA was not affected by ADA, unlike other adenosine-based NRTIs, such as EdA; while EdDAP was deaminated to EdG, whose anti-HIV-1 activity was identical to that of EdDAP, indicating that the activity of EFdA and EdDAP is not affected by ADA. The pharmacodynamics elucidated here should contribute to the sustained potency of EFdA and EdDAP. [Conclusion] We believe that EFdA and EdDAP should serve as potential candidates as next-generation \(qd\) NRTIs that are efficacious in patients harboring both wild-type and resistant variants.

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GRL-007: a novel small molecule CCR5 antagonist poten against a wide spectrum of HIV-1
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CCR5 is a member of the G-protein coupled receptor family and serves as an essential co-receptor for cellular entry of R5-tropic HIV-1. Thus, CCR5 represents a validated target for the therapeutics against HIV-1 infection. However, as of today, maraviroc (MVC) is the only CCR5 antagonist in clinical use and certain strains of HIV-1 have developed resistance against MVC. Thus, development of CCR5 antagonists with a novel scaffold(s), that are potent against drug-resistant HIV-1 strains is urgently needed. In this study, we designed, synthesized, and characterized GRL-007 (G-007), a novel CCR5 antagonist, containing a unique piperazine scaffold. G-007 had an antiviral activity (IC\(_{50}\)) of
1.4 nM against wild-type HIV-1 in MAGI assay, and the activity was comparable to that of MVC (0.7 nM). Against HIV-1 variants resistant to two experimental CCR5 antagonists, AD101 and vicriviroc, G-007 had a 3.8- and 5.3-fold change in activity relative to the wild-type HIV-1, whereas MVC had a 14.8- and 10.6-fold change in activity, respectively. We elucidated the binding mode and interactions of G-007 with CCR5 by homology modeling and molecular docking. The model suggests that G-007 binds in the hydrophobic cavity of CCR5 formed within the upper transmembrane domains and at the second extracellular loop (ECL2) of CCR5. Several important interactions of G-007 with CCR5 residues have been identified, including Cys-178 located in ECL2 and Glu-283 located in transmembrane-7 of CCR5. We further probed the binding site of G-007 by competitive assay against various CCR5 antibodies. The data suggest that G-007 interacts with the ECL2 the same as other reported CCR5 antagonists. On the other hand, G-007 only partially blocked (~50%) the binding of CCR5 to RANTES, the natural ligand of CCR5, whereas most of the other CCR5 antagonists almost completely blocked the RANTES binding. G-007 also demonstrated similar behavior in the inhibition of RANTES-induced Ca²⁺ flux. Taken together, our data suggest that G-007 might represent a potent anti-HIV-1 agent with only partial interactions with RANTES and warrant further development as a potential therapeutic option for RS tropic HIV-1.

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Ligand-Bound Structures of the Dengue Virus Protease Show the Active Conformation.

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Dengue is a mosquito-borne virus that is a major threat to human health in tropical and subtropical regions. We have solved two crystal structures of the dengue virus serotype 3 (DENV-3) protease, bound to inhibitors. In the presence of an aldehyde-peptide, the DENV-3 protease forms the closed conformation in which the β-hairpin region of NS2B wraps around the NS3 protease core, in a similar manner to the West Nile virus protease. Our results show that other flavivirus proteases form the ‘closed’ conformation. In addition, we have solved the structure of the DENV-3 protease bound to the serine-protease inhibitor, aprotinin. Aprotinin binding by DENV protease does not require interactions with NS2B. Our structures will allow rational, structure-based design approaches against this important anti-dengue target.

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Establishment of a highly sensitive detection system for HIV-1 RNA in saliva using sugar-immobilized gold nanoparticles

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Background: The current standard method for diagnosis of HIV-1 infection has to use a blood sample. However, there are a substantial number of HIV-1-infected individuals who cannot access to the equipments for collecting and transporting blood samples. Saliva is considered as an alternative specimen for the diagnosis of HIV-1 infection, since it contains a small amount of HIV-1 in patients. In this study, we have established a simple, accurate, and sensitive system for detecting HIV-1 in saliva using gold nanoparticles that immobilize sugars specifically interacting with virus particles. Methods: The sugar-binding properties of HIV-1 were analyzed by a
surface plasmon resonance system equipped with gold-coated chips immobilizing various sugar-chains (Sugar-Chip). The sugar chains that bound to HIV-1 were immobilized onto the nanoparticles and mixed with diluted virus suspension. The nanoparticle-binding HIV-1 was subjected to real-time reverse transcription PCR after concentration. **Results:** Irrespective of co-receptor usage of HIV-1, heparin showed the strongest binding affinity to HIV-1 among the forty-seven sugar-chains on Sugar-Chip. Therefore, the heparin-immobilized gold nanoparticles (Heparin-GNP) were used for concentrating HIV-1 in saliva. Heparin-GNP successfully concentrated virus particles in saliva from a healthy donor and markedly increased the sensitivity of the detection system to HIV-1. After concentration of HIV-1 in saliva from patients with Heparin-GNP, the sensitivity of the system was comparable to that of a standard clinical method for plasma samples without concentration. **Conclusions:** These results indicate that the present method is a simple and effective tool for screening HIV-1 infection.

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**Establishment of in vitro culture system for hepatitis E virus (genotype 3) originating from human blood plasma and its characterization**

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[Background] The hepatitis E virus (HEV) has been considered to be water-borne, mostly found in developing countries. However, recently, HEV has been found to be spreading worldwide including in industrialized nations. HEV was demonstrated to be transmitted by blood transfusion and to occasionally cause severe hepatitis. The purpose of this study is primarily to establish a quantification system for HEV infectivity that would be applicable to evaluating strategies for ensuring blood product safety.

[Materials and Methods] Fourteen plasma or serum samples containing HEV were used. Two cell lines, PLC/PRF/5 and A549, were inoculated with these samples. Viral culture medium was changed to a fresh one every week. The number of HEV RNA copies was determined by real-time RT-PCR. The efficacy of the Mirasol Pathogen Reduction System (CaridianBCT) using Riboflavin + UV rendering viral genome damaging against HEV infectivity was investigated following the manufacturer’s instructions. **[Results and Discussion]** Viral progenies were detected in recovered media obtained from two of 14 inoculation series. One of them, designated as JRC-HE3, was cultured for 4 passages. The copy number of HEV was completely the same in all passages. Viral limiting dilution assay demonstrated that an infectious titer, 1 TCID, corresponded to $10^{3.5}$ copies. A morphological analysis of HEV showed numerous round particles with a diameter of approximately 30 nm. These particles were tentatively proven to be HEV virions by an immunological method. Full-genome sequencing of JRC-HE3 obtained from both plasma and the progenies incubated for 150 days was carried out to investigate genomic alterations which revealed few variations. More than a 2 log decrease in the titer of infectious HEV in a blood component of platelet concentrate was obtained following Mirasol. Using this culture system, the precise mechanisms underlying HEV infection and anti-HEV activity of the Mirasol are currently being investigated.

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**Combination of triterpenoids from Platycodon grandiflorum with Interferon-α enhanced suppression of hepatitis C virus replication in vitro and in vivo**
Hepatitis C virus (HCV) afflicts approximately 200 million people worldwide. Currently direct-acting antiviral agents (DAAs) provide promises of clinical efficacy, but are possibly hampered by significant adverse effects and emergence of resistant strains. We found a potent inhibitor from extracts of *Platycodon grandiflorum* (PG) that inhibited HCV RNA replication in Huh7 cells harboring HCV replicon. Six triterpenoids (PD, PD2, PD3, DPD, PD4, and PA) were identified as active components and these triterpenoids exerted RNA-dependent RNA polymerase inhibition activity and enhancement of the anti-HCV effect of IFN by up-regulation of IFN-stimulated genes (ISGs), such as oligoadenylate synthetase (OAS) 1 and ISG15. In the present study, we investigated the synergistic antiviral activity of PG-extract ("Dr. J" as health food) in combination with IFN-α both *in vitro* and *in vivo*. Combination Index (CI) values in combination of PG-extract with IFN-α, other DAAs were calculated <1.0 (from additive to synergistic) by CalcuSyn (Biosoft, UK). Colony forming assays were analyzed to determine the degree to suppress the development of resistance in GT 1b replicon, PG-extract alone or in combination with IFN-α, other DAAs. Suppression of replicon bearing cells was found to be greater in combination than any other agent on its own. "Dr. J" showed efficacy of >5.0 Log reduction in HCV RNA within 12weeks, when used in combination with IFN-α/RBV in a small number of chronically HCV-infected patients. In conclusion, triterpenoids extracted from PG efficiently represent novel anti-HCV therapeutic approaches to suppress HCV replication and drug resistant mutations.

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Characterization of PG 301029-Resistant Cells and Anti-HCV Activity of PG301029 Against Resistant HCV Replicons

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PG 301029 inhibits HCV viral RNA production from replicon cells through a novel mechanism. Characterization of PG 301029-resistant cells and activity of PG301029 against resistant replicons was performed to further define the antiviral activity of the compound. PG 301029-resistant replicon cells were selected in the presence of G418 and PG 301029. Resistant cells had a 20-fold decrease in sensitivity to PG 301029, ~5-fold cross-resistance to BMS 790052, and were hypersensitive to VX-222 and IFN-a2B. Mutations NS3 D168N and NS5A C446R were identified in replicons recovered from resistant cells and introduced into parental replicon plasmid sequences for *in vitro* transcription and electroporation of Huh/luc-net cells. Individual NS3 or NS5A mutations or the combination of both mutations conferred a 3-fold, no change, and 9-fold decrease in sensitivity to PG 301029, respectively. Analysis of the replication of wildtype HCV in PG 301029-cured cells selected by culture of replicon cells in the presence of PG 301029 for 27 days and Huh/luc-net cells demonstrated that HCV replication was reduced >2-fold in the cured cells. Cross-resistance analysis revealed that PG 301029 was equally effective in the inhibition of HCV replicons containing NS3 A156T or D168V, conferring resistance to telaprevir or boceprevir, respectively, as it was in inhibiting wildtype sequences. PG301029 resistant cells were not significantly cross-resistant to various classes of HCV inhibitors, suggesting a novel mechanism of action for PG 301029. The observations that the double mutation does not restore maximal resistance of the selected cells and that cured cells demonstrated a reduced capacity for the replication of wild type HCV replicon, indicates that prolonged exposure to PG 301029 alters host cell functions necessary for efficient viral replication. The location of the mutations in the resistant replicon and hypersensitivity to VX 222 and IFN-a2B suggests that PG 301029 may impact NS5A and or NS5B-containing proteins. The lack of decrease in sensitivity of HCV replicon sequences containing mutations in NS3 known to
confer resistance to telaprevir and boceprevir suggests that this class of compound may have beneficial therapeutic value in combination treatments.

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Preclinical Characterization of Miravirsen (MIR), a Novel anti-HCV Therapeutic Targeting the Host Cell Factor miR-122
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Background and aims: MIR is a β-D-oxy-locked Nucleic Acid modified phosphorothioate anti-sense oligonucleotide inhibitor of the liver-expressed microRNA-122 (miR-122). miR-122 binds to two closely spaced target sites (S1 and S2) in the 5'-untranslated region (UTR) of the HCV genome, and forms an oligomeric miR-122-HCV complex, thereby protecting the 5'-HCV genome from nucleolytic degradation (Machlin et al. PNAS 2011). In vitro, MIR has demonstrated broad spectrum of activity against chimeric replicons carrying the 5'-UTR from each of the six main HCV genotypes (Li, PNAS 2011). Here we present the further characterization of the in vitro antiviral activity and resistance profile for MIR. Methods: In vitro antiviral activity, selectivity, serum interference, resistance, and combination studies were performed in the genotype 1b HCV replicon system. All studies with MIR were performed without the use of a transfection agent. Results: MIR demonstrated antiviral activity against HCV genotype 1b with a mean EC50 value of 0.671 μM (±0.327) and an EC90 value of 5.4 μM (±3.23). No cytotoxicity was observed up to the highest concentration tested (>320 μM) in a variety of different cell culture models including Huh-7 cells, primary hepatocytes, stimulated and unstimulated PBMCs, macrophages, bone marrow cells, TK-10 and HepG2 cells yielding a therapeutic index of ≥297. Antiviral activity was not reduced in the presence of 40% human serum, 45 mg/mL human serum albumin or 1 mg/ml alpha-1-acid glycoprotein. Combination studies of MIR with IFN alpha 2b, ribavirin, non-nucleoside and nucleoside inhibitors of NS5B (VX-222, 2'Me-C), NS5A (BMS 790052), or NS3 (telaprevir) indicated additive interactions. A 25-day treatment with MIR up to 20x EC50 did not lead to the emergence of resistant colonies. In contrast, replicon treated with telaprevir rapidly gave rise to resistant colonies. Conclusions: MIR has demonstrated potent in vitro antiviral activity, an additive effect with all HCV antivirals tested and a high barrier of resistance. This data set supports the continued clinical development of MIR in HCV infected individuals.

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Antiretroviral agents effectively block HIV replication after cell to cell transfer
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Cell to cell transmission of HIV has been proposed as a mechanism contributing to virus escape to the action of antiretrovirals and a mode of HIV persistence during antiretroviral therapy (Sigal et al. Nature 2011). It has been postulated that the drug concentration required to prevent a single transmitted virion from infecting a target cell is much lower than that needed to stop multiple transmitted virus particles from infecting the same cell in cocultures of infected and uninfected cells. Here, cocultures of infected HIV-1 cells with CD4+ cells were used to evaluate virus transmission. Analysis of capsid antigen (p24) transfer, qPCR of proviral DNA and integrated DNA and measurement of p24 in the supernatant of infected cells were used to evaluate virus replication in the absence or presence of antiretroviral drugs at different time points. Coculture of HIV-1 infected cells with CD4+ T cells led to
The broad spectrum antiviral activity of T-705 is extended to norovirus
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Human noroviruses are a primary cause of gastroenteritis and the main cause of foodborne illness throughout the world. Despite this, there is still no antiviral drug available today for treatment or prophylaxis of norovirus disease. The antiviral compound T-705 (favipiravir) has previously shown broad-spectrum activity against RNA viruses such as influenza and arenaviruses by presumably targeting the viral RNA polymerase after conversion to its active form T-705 ribofuranosyl triphosphate. In the present study, the anti-norovirus activity of T-705 was evaluated through an in vitro colorimetric assay (MTS/PMS) based on the reduction of virus-induced cytopathic effect (CPE). This screening assay used infectious murine norovirus (MNV) as a surrogate for human norovirus. The antiviral activity of T-705 was also tested by assessing its effect on viral RNA synthesis by qRT-PCR. T-705 inhibited norovirus replication in a concentration-dependent manner both when assessing its effect in virus-induced CPE (EC50=39.3 μg/mL [250 μM]) and in viral RNA synthesis (EC50=19.5 μg/mL [124 μM]). Despite its moderate activity, T-705 was able to fully inhibit norovirus replication at 100 μg/mL [637 μM] with negligible cytotoxicity. Time-of-drug addition studies indicated that T-705 could interfere directly with RNA synthesis at the level of the MNV polymerase. The present work showed that the broad spectrum of activity of T-705 is extended to norovirus. Hence, it becomes relevant to elucidate the mechanism of action underlying the anti-norovirus activity of T-705 and how it compares to what has been described to other RNA viruses. Acknowledgments. FCT (Fundação para a Ciência e a Tecnologia) for the PhD grant of J. Rocha-Pereira (SFRH/BD/48156/2008) Herbert W. Virgin for the generous provision of the MNV

Design, Synthesis and Assay of Novel Mercaptobenzimidazole Derivatives Against the West Nile Protease Target
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Background: The mosquito-borne viral pathogens of global significance include the members of flavivirus genus of Flaviviridae family. Two important human pathogens are dengue and West Nile viruses which cause considerable morbidity and mortality throughout tropical and subtropical regions of the world. No vaccines or antiviral therapeutics are available for these two pathogens. The overall goal of our study is to develop potent inhibitors of West Nile virus serine protease, which is an excellent viral target as it is required for viral replication. In this study, we examined whether derivatives of Mercaptobenzimidazole could be versatile lead compounds for structure-activity relationship study. Methods: Novel Mercaptobenzimidazole derivatives synthesized and screened for their inhibitory activities of WNV protease in vitro. Molecular modeling was performed by computational methods to understand mode of action of the compounds. Results: The N-Sulphanomidomethyl-Mercaptobenzimidazole (MBZ-SN) exhibited significant inhibitory activities against the WNV protease (IC₅₀ values of 2.5 µM). Modeling suggests that MBZ-SN could bind at the active site of WNV protease although co-crystallization of the viral protease with the active compound is required to confirm the modeling data. Free – SO₂NH₂ group is essential for activity and any substitution decreases the inhibitory activity (for example, MBZ-SDM and MBZ-SAC). Conclusions: To our knowledge, this is the first report regarding the inhibitory activities of Mercaptobenzimidazole derivatives against the WNV serine protease. Further work on SAR study for lead optimization is in progress.

Studies of Dengue NS3 Protease Inhibitory Activity of Novel Isatin Derivatives

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Background: The mosquito-borne viral pathogens of global significance include the members of flavivirus genus of Flaviviridae family. Two important human pathogens are dengue and West Nile viruses which cause considerable morbidity and mortality throughout tropical and subtropical regions of the world. No vaccines or antiviral therapeutics are available for these two pathogens. The overall goal of our study is to develop potent inhibitors of Dengue virus serine protease, which is an excellent viral target as it is required for viral replication. In this study, we examined whether derivatives of isatin (2, 3-dioxoindole) could be versatile lead compounds for structure-activity relationship (SAR) study. Methods: Novel isatin-sulphadimidine derivatives were analyzed for their inhibitory activities of Dengue NS3 protease in vitro. Results: 5-chloro-N-acetyl derivative (SPIII-SCI-AC) exhibited significant inhibitory activities against the dengue NS3 protease (IC₅₀ values of 9.4 µM). Conclusions: To our knowledge, this is the first report regarding the inhibitory activities of isatin derivatives against the Dengue serine
protease. Further work on SAR study for lead optimization is in progress. Inhibitory activity of Isatin Derivatives against Dengue NS3 Protease

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<th>COMPOUND</th>
<th>% INHIBITION</th>
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STUDIES ON HIV INTEGRASE AND HIV INTEGRASE/LEDGF INHIBITORY ACTIVITY OF ETHANOLIC FRACTIONS (F1-F6) OF MORINDA CITRIFOLIA L NONI

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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 HIV integrase and HIV Integrase/Lens Epithelium Derived Growth Factor (LEDGF) inhibitory activity of different fractions (F1-F6) of ethanolic extract of Morinda citrifolia METHOD: Various fractions (F1-F6) of ethanolic extract of Morinda citrifolia fruit have been studied against inhibition of HIV-1 integrase enzymatic activity by oligonucleotide based assay and HIV IN/LEDGF-P75 assay performed by Alpha Screen Technology, respectively. All fractions of Morinda citrifolia were investigated for both 3’processing and strand transfer process of HIV-1 integrase enzymatic activity. RESULTS: All fractions except F1 exhibited inhibitory activity against HIV-1 integrase enzyme (3’P IC50: 30-73 μg/ml and ST IC50: 4-56 μg/ml). The F2 fraction displayed significant inhibitory activity against both step of HIV IN enzymatic activity (3’P IC50:4±1 μg/ml and ST IC50:49±15 μg/ml) and F3 fraction inhibits the HIV IN/LEDGF interaction at the concentration of 50 μg/ml. CONCLUSION: Anthroquine, flavanoids and glycosides are the principle active constituents of different ethanolic fractions of Morinda citrifolia, which may responsible for HIV integrase inhibitory activity. HIV INTEGRASE INHIBITORY ACTIVITY OF MORINDA CITRIFOLIA L
Arenaviruses are enveloped viruses containing a bipartite, single-stranded RNA genome, with ambisense coding strategy. Five arenaviruses cause severe hemorrhagic fevers in humans, but at present no reliable drug therapy is available. The presence in arenaviruses of the Z protein, containing a highly conserved RING finger motif, prompted us to initiate studies about this protein as a possible target for a viral inhibitory strategy. We have previously shown that antiretroviral compounds with diverse chemical structures, kindly provided by the National Cancer Institute (USA), which target to the Zn-finger motifs in the HIV nucleocapsid protein NCp7, display antiviral and virucidal activity against arenaviruses. Here, the in vitro inhibitory activity of a selected group of aromatic disulfides with diverse substitutions is reported. The carboxamide-derivatized disulfide NSC4492 and the amino-nitro-derivative NSC71033 demonstrated moderate antiviral activity against the pathogenic arenavirus Junín (JUNV) as determined by virus yield inhibition assay in Vero cells, with values of antiviral effective concentration 50% (EC50) in the range 27.7-32.4 µM, but a very potent virucidal effect, with inactivating concentration 50% (IC50) in the range 0.2-0.5 µM. NSC4492 inactivated diverse arenaviruses, with a linear kinetics of reaction in a temperature dependent form. In addition, the activity spectrum of these disulfides also included viruses from other families, but the reactivity was greater against those viral agents containing RING finger motifs like JUNV. Mechanistic studies demonstrated that viral RNA synthesis was blocked after infection of Vero cells with inactivated JUNV virions, with subsequent inhibition of viral protein expression. Furthermore, the interaction of the disulfide with JUNV Z protein was determined by Western blot analysis of virus-like particles released into the supernatants from cells expressing an HA-tagged version of Junin virus Z protein in presence of NSC4492. An alteration in the electrophoretic profile of Z oligomers was observed, suggesting that the compound might induce conformational change in Z protein.

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**Inactivation of arenavirus infection by aromatic disulfides**

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**Mammalian cells persistently infected with Japanese encephalitis virus return to normal phenotype on curing with siRNA**

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Small interfering RNA (siRNA) mediated inhibition of virus replication has been reported for a variety of viruses. Here we report complete curing of porcine kidney (PS) cells that were persistently infected with Japanese
encephalitis virus (PI-JEV) by RNA interference. The PI-JEV cells have been maintained for over 130 passages and continuously shed infectious JEV. Three synthetic double-stranded siRNAs targeted to core, envelope and NS5 regions of JEV genome were used to interfere with ongoing JEV replication in the PI-JEV cells. Transfection of PI-JEV cells with siRNA to core and the mixture of three siRNAs completely abolished JEV antigen production. However, this effect was transient. We then cloned the cells transfected with the mixture of three siRNAs and obtained two clonal cell lines (PIC-3 and PIC-5) that were negative for viral antigen, viral RNA and infectious JEV. The PI-JEV cells were refractive to super-infection with flaviviruses, large in size, slow growing, abnormal in actin distribution (Figure 1) and showed cytoplasmic inclusion bodies with disrupted Golgi. However, on curing, the PIC-3 cells exhibited several characteristics that were similar to normal parental PS cell phenotype in that they 1) regained the susceptibility to flaviviruses 2) were similar in size as normal PS cells 3) had doubling time similar to normal PS cells 4) had normal distribution of filamentous actin(Figure 1) 5) were devoid of inclusion bodies and had normal Golgi complex morphology. All these studies indicated that siRNA treatment was not only effective in curing the long-term JEV persistence but the cured cells showed several characteristics similar to normal uninfected cells. Figure1: Organization of F actin in (A)PS(B)PI-JEV (C)PIC-3 cells:

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Influenza Virus Infections in Mice are Exacerbated by Intranasal Drug Delivery and are Difficult to Treat with Zanamivir
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Compounds lacking oral activity are often delivered intranasally (i.n.) to treat influenza virus infections in mice because inhalation may become the treatment route for human use, as occurred with Relenza® (zanamivir). However, i.n. treatments can greatly enhance the virulence of virus infections. In order to not overwhelm the drug with too severe of an infection, lethal challenge dose titrations coupled with i.n. liquid (placebo) treatments should first be performed to select proper virus challenge doses. We found that influenza A (H1N1, H3N2, and H5N1) virus challenge doses when followed by i.n. treatments can be 100-10,000 fold lower than exposures without such treatment and yet cause equivalent mortality. An analysis of virus production following low virus exposures coupled with intranasal placebo treatments produced low virus titers on the first day of infection, but by day 3 mouse lungs had equivalent lung virus titer compared to lungs exposed to higher challenges without i.n. liquid treatment. By day 5, lung hemorrhage scores and lung weights in mice treated by i.n. route reached the same high levels as those obtained in mice given higher virus challenges without i.n. liquid. Low virus exposures without liquid treatment were non-lethal. The exacerbating effects of the i.n. liquid reduces antiviral drug efficacy. Here we demonstrate that zanamivir is 100% effective at 10 mg/kg/day by oral, intraperitoneal, and intramuscular routes against high influenza A/California/04/2009 (H1N1) virus challenges in mice. However, the compound administered i.n. at 10-20 mg/kg/day gave no protection from death from infections initiated by a low virus inoculum (exacerbated by i.n. drug or placebo treatments), although statistically significant delays in the time to death occurred. This presents challenges for the evaluation of compounds tested by i.n. route because of the
severity of the infections. Other virus strains may be more appropriate for testing of compounds by i.n. route, however. Mice lethally infected with influenza A/Victoria/3/75 (H3N2) were protected by i.n. zanamivir treatments. [Supported in part by contract N01-AI-30063 (Awarded to Southern Research Institute, Birmingham, AL) from the Virology Branch, NIAID, NIH]

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Evaluation of Influenza Virus Endonuclease Inhibitors by Cell Culture and Enzymatic Methods, Including a Novel Real-Time Fluorescence Assay
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The influenza virus PA endonuclease cleaves host pre-mRNAs to generate capped primers for viral mRNA synthesis. This 'cap snatching' activity is a prime target for antiviral therapy. Reported endonuclease assays using gel electrophoresis are discontinuous and time-consuming. We describe a novel method using molecular beacons (MB) as a substrate. MB cleavage by recombinant PA-Nter (i.e. residues 1-220 of PA) separates the fluorophore from the quencher, and the evolving fluorescence can be monitored in real-time. Since the substrate preference of PA is unclear, we compared different MBs, and found that a U-rich MB substrate (MB-U) is preferred over an A- or C-rich MB. Gel electrophoresis showed virtually complete digestion of 20 nM MB-U within 90 min incubation with 1 µg PA-Nter. In real-time assays (20 nM MB-U, 1 µg PA-Nter, 384-well plate), fluorescence was maximal at 20 min, with a signal-to-noise ratio of 16. A linear relationship was observed between initial cleavage rate and enzyme or MB-U concentration, indicating Michaelis-Menten kinetics. Two known diketo acid inhibitors of PA, i.e. 2,4-dioxo-4-phenylbutanoic acid (DPBA) and L-742,001, inhibited the MB-U cleavage by PA-Nter in a concentration-dependent manner. Next, we determined the activity of DPBA, L-742,001 and ribavirin in two cell culture assays, i.e. a virus yield assay, based on RT-PCR quantification of influenza virus released from MDCK cells at 24 h pi, and a luciferase-based viral ribonucleoprotein (vRNP) reconstitution assay in 293T cells. For both L-742,001 and ribavirin, the EC50 values were similar in the two assays. DPBA was inactive in cell culture, possibly due to low intracellular uptake. Whereas the vRNP assay is appropriate for cell culture screening of potential PA inhibitors, our novel MB enzymatic assay is well suited to study their inhibitory effects at the biochemical level.

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Heat shock protein 70 inhibits HIV-1 Vif-mediated ubiquitination and degradation of APOBEC3G
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The cytidine deaminase APOBEC3G, which is incorporated into nascent virus particles, possesses potent antiviral activity and restricts Vif-deficient HIV-1 replication at the reverse transcription step through deamination-dependent and -independent effects. HIV-1 Vif counteracts the antiviral activity of APOBEC3G by inducing
APOBEC3G polyubiquitination and its subsequent proteasomal degradation. Heat shock proteins play critical roles in the life cycle of a variety of RNA and DNA viruses. For example, heat shock protein 70 (HSP70) is specifically incorporated into HIV-1 virions. However, the formation of the P-TEFb/Tat/TAR complex is required to stabilize the CDK9/cyclinT1 heterodimer by HSP70 and HSP90.

To better develop potential novel therapeutic strategies to exploit APOBEC3G’s antiviral function, we investigated the role of HSP70 in APOBEC3G function. We found that siRNA against HSP70 significantly reduced the level of APOBEC3G in the presence of HIV-1 Vif, but not in the absence of Vif. In addition, overexpression of HSP70 in 293T cells reduced the Vif-mediated degradation of APOBEC3G by inhibiting APOBEC3G polyubiquitination. This effect is attributed to the impairment of APOBEC3G-Vif binding. Furthermore, overexpression of HSP70 in the presence, but not in the absence, of APOBEC3G clearly suppressed the infectivity of virions in a dose-dependent manner. These results suggest that HSP70 acts as a potential antiviral host factor through interaction with APOBEC3G and may form the basis for new anti-HIV-1 therapies.

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Cepharanthine and a tetramethylnaphthalene derivative synergistically inhibit HTLV-1-infected cell proliferation in vitro

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The novel tetramethylnaphthalene derivative TMNAA was previously found to selectively inhibit the proliferation of HTLV-1-infected T-cell lines but not HTLV-1-uninfected T-cell lines. Since TMNAA did not affect NF-κB activity, TMNAA was examined for its anti-proliferative activity various T-cell lines in combination with cepharanthine (CEP), which is known to inhibit NF-κB. HTLV-1-infected and uninfected T-cell lines were cultured in the presence of various concentrations of TMNAA and CEP, and their proliferation and viability were determined by a tetrazolium dye method. The mode of cell death was also examined by flow cytometry and Western blot analysis. The 50% inhibitory concentrations (IC50) of TMNAA and CEP for the ATL cell line (S1T) were 1.65 ± 0.03 and 1.97 ± 0.29 µM, respectively. On the other hand, the IC50 of TMNAA and CEP combination (1:1) resulted in 0.93 ± 0.13 µM, indicating that the combination synergistically inhibited the proliferation of S1T cells. Such synergism was observed for another infected cell line (MT-2) but not for the HTLV-1-uninfected cell lines MOLT-4 and CEM. Moreover, TMNAA did not induce apoptosis of S1T cells, but CEP did. Interestingly, TMNAA significantly enhanced the CEP-induced apoptosis of S1T and MT-2 cells. Consequently, the combination of TMNAA and CEP strongly and selectively inhibits the proliferation of HTLV-1-infected cell lines through the induction of apoptosis. Therefore, TMNAA and CEP may have potential for chemotherpay of ATL.

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Molecular Modelling studies on DENV helicase.

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Dengue virus (DENV) is a mosquito-borne virus that belongs to the Flaviridae family and that is endemic in over 100 countries. Every year, it causes approximately 50-100 million new infections in humans. There is currently no specific antiviral treatment available and, to date, vaccine development has proven very difficult. Three distinct
clinical pictures have been described for DENV infection: dengue fever, dengue haemorrhagic fever and dengue shock syndrome. The latter two are commonly caused by a secondary infection with another DENV serotype and more frequently have a fatal outcome. The viral single-stranded, positive-sense RNA genome encodes three structural and seven non-structural (NS) proteins. The latter are viral enzymes and cofactors, and therefore attract significant attention for the development of selective inhibitors of virus replication. One such promising target is the NS3 NTPase/helicase as it is crucial for unwinding of the dsRNA intermediate, an essential step during RNA replication. Based on available crystallographic data, we have applied different molecular modelling techniques to investigate the nucleic acid binding site of NS3 and to search for potential inhibitors of this protein. Starting from a database of approximately 200,000 molecules, a virtual screening approach allowed selection of drug-like compounds for in vitro evaluation. Furthermore, with the aim of identifying a novel target sub-site within the RNA binding pocket of NS3, we have performed a series of molecular dynamic simulations to gather further insights in the RNA-enzyme interactions. In this presentation, we will discuss the preliminary results of these studies and the potential implications in antiviral drug design against this viral target.

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HCV RdRp-Complex Simulation for the Understanding of Recognition Element Mediated by Pseudonucleoside Inhibitor

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A worldwide research is underway to discover HCV RNA dependent RNA polymerase (RdRp) inhibitor as direct acting antiviral (DAA). A number of RdRp inhibitors have progressed into the clinical phases and have demonstrated proof-of-concept by reducing viral loads in HCV infected patients. Unfortunately, there are no complete crystallographic ternary complexes of HCV RdRp with NTPs are yet publically known to describe the initiation or elongation function. The slow drug development against HCV may attribute due to poor 3D structural information of this key enzyme. Therefore, the present investigation started with the development and calibration of a complete structural model of the replicase complex (RdRp, metal ions, short chain of template and primer, including incoming NTP) using homology & superposition followed by global minimization, validated by experimental facts to expedite anti-HCV research. The molecular dynamics simulation studies with several potential pseudonucleoside reveals the molecular recognition motif of RdRp, which describes the conformational requirement of pseudonucleoside and its interaction with hot spot residues of RdRp. The molecular docking and dynamics simulation of most potential compounds (2'- and 4'-altered pseudonucleoside) reveals the binding mode differences from natural substrate. Further, the dynamics studies highlight the molecular basis of most prominent mutant S282T and S96T in response to 2' and 4' alteration respectively. Overall the present study opens a structure model of RdRp-inhibitor complex of wild type as well as with prominent mutant towards identifying a novel inhibitor with safe profile including drug like properties.
Interaction model of HIV-1 Rev and a Rev-multimerization inhibiting nanobody

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HIV-1 Rev is the key regulator for nucleocytoplasmic export of viral mRNAs via the CRM1-dependent transport pathway. Despite many efforts to inhibit its function, no Rev inhibitors made it into clinical trials yet. While most attempts to tackle Rev function focused on the Rev-RNA and Rev-CRM1 interaction, we recently identified a llama single-chain antibody (Nb₁₉₀) as the first inhibitor targeting the N-terminal Rev multimerization domain. This nanobody showed to be a potent intracellular antibody in that it efficiently inhibits HIV-1 viral production. In order to gain structural insight into the Nb₁₉₀-Rev interaction interface, we performed mutational studies to map the nanobody paratope. Alanine mutants of the hypervariable domains of Nb₁₉₀ were evaluated in different assays measuring Nb₁₉₀-Rev interaction and viral production. Eight residues within Nb₁₉₀ were found to be crucial for epitope recognition. These experimental data were used to perform docking experiments and map the Nb₁₉₀-Rev structural interface. The model reveals that four of the selected amino acids (T33, F100, F105 and D107) make direct contacts with two Rev epitope residues (K20 and Y23). Three other residues (F50, N96, D98) stabilize the CDR3 loop structure of ND₁₂₀⁰, while the M34 residue is important for overall nanobody structure. This Nb₁₉₀ paratope mapping model can be applied for the rational development of smaller entities binding to the Nb₁₉₀ epitope, aimed at interfering with protein-protein interactions of the Rev N-terminal domain.

Dengue virus infection of human dermal microvascular endothelial cells is inhibited by sulfated Escherichia coli K5 polysaccharide derivatives.

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Dengue virus (DENV) infection may cause dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), which is characterized by an increase in vascular permeability and plasma leakage. Dendritic cells are the main target for DENV infection in vivo, but endothelial cells (ECs), which constitute the primary fluid barrier of the vasculature, may be infected by DENV as well. We found that the human dermal microvascular endothelial cell line (HMEC-1) is permissive to DENV-2 infection and replication, as demonstrated by anti-DENV specific mAbs and quantitative rTPCR. HMEC-1 cells express various markers of ECs but did not express the cognitive DENV receptor Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN). However, heparan sulfate proteoglycans (HSPGs) were highly expressed on these cells and treatment of the cells with soluble heparin, heparan sulfate or chondroitin sulfate A and B reduced DENV infectivity by 64-90%, suggesting that DENV interferes with HSPGs on HMEC-1 cells. The capsular K5 polysaccharide of E. coli has the same structure as the biosynthetic precursor of heparin. Therefore, we tested the capacity of various sulfated K5 derivatives to inhibit DENV replication. In contrast to heparin, these compounds are devoid of anticoagulant activity and their antiviral activity against human immunodeficiency virus and herpes simplex virus has already been demonstrated. None of the compounds proved toxic for HMEC-1 cells at the highest concentration tested (3 µM). Two K5 polysaccharide derivatives, the highly sulfated K5-OS(H) (11 kDa) and K5-N,OS(H) (15 kDa), dose-dependently inhibited DENV replication (EC₅₀ of 111 nM and 107 nM respectively). When HMEC-1 cells were pretreated with different concentrations of these compounds and washed before exposure to DENV no inhibitory activity was observed, indicating that these highly sulfated K5 derivatives do not interact with cellular membrane proteins. Experiments
are ongoing to determine whether the K5 derivatives interfere with the attachment or fusion process of DENV to HMEC-1 cells and whether they are able to inhibit DENV-induced EC permeability in vitro.

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Analysis of HIV-1 drug resistance-associated mutations in treatment-naïve individuals circulating in Liaoning from 2004 to 2010
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OBJECTIVE: To update the baseline surveillance data of HIV-1 drug resistance associated mutations in treatment-naïve individuals circulating in Liaoning, this study evaluated the development of resistance mutations and examined the susceptibility of HIV with these mutations to antiretroviral in treatment-naïve individuals before their therapy regimes. METHODS: RNA was extracted from 13 plasma samples of diagnosed untreated HIV-1-infected treatment-naïve patients. The protease and nucleoside reverse transcriptase coding regions were amplified by RT-PCR, nested PCR and sequenced directly. Levels of resistance were evaluated according to the Stanford University HIV Drug Resistance Database’s algorithm (http://hivdb.stanford.edu). RESULTS: An overall prevalence of 30.8% (4/13) resistance to RTI and 7.7% (1/13) to PRI. The most frequent substitutions in the RT region were at positions P225H, K238S, V179D, and K238T. A71V substitution in PR was found in 6 samples, but no any worse with drug sensitivity. Major position I54S in PR implied to a multiple drug-resistance. Polymorphisms in subtype A, H and circulating recombinant forms (CRFs) CRF10-CD sequences were identified. CD4 and Viral Loading are negative correlation (r=-0.165), but P=0.295, no significant difference. CONCLUSION: This is the first study reporting the higher prevalence and more patterns of both PRI and RTI resistance-associated mutations in naive HIV-1 infected patients after 2004 circulating in Liaoning. These data underline the importance of genotypic resistance testing of chronically HIV-1-infected patients before initiating treatment, in order to select the most suitable drug regimen. [Key words] treatment-naïve; HIV-1 infected subjects; Drug resistance-associated mutations

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Impact of Viral Sequences beyond HCV NS5A domain I on Potency of HCV NSSA inhibitors
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Background: As a component of viral replication complex, HCV NS5A consists of 3 major domains, thought to be involved in viral RNA replication (domain I & II) and virus production (domain III). Domain I is believed to be involved in the antiviral activity of NSSA inhibitors since the resistance mutations are mapped to that domain. In many cases, the potency of these inhibitors against various HCV strains is determined by replacing only the domain I sequence of a genotype-1b-based replicon with that from the strain. Knowing that the function of NS5A and the mechanism of action of NSSA inhibitors are still unclear, we examined the impact of the remaining viral sequences particularly the sequences of domain II & III of NS5A on the antiviral activity of NSSA inhibitors. Methods: A transient HCV GT-1b replicon was used for construction of chimeric replicons with the first 100 amino acids or the entire NS5A replaced by the corresponding NS5A region from GT-1a/H77 or J1 strain or GT-2a/JFH-1 strain. Antiviral activity of an NSSA inhibitor BMS-790052 was evaluated after transfection of these chimeric replicons into Huh-Lunet cells. For comparison, the antiviral activity of BMS-790052 was also evaluated in cell lines carrying either the full-length GT-1a/H77 or 2a/JFH-1 non-chimeric replicon. Results: Similar EC50 values of BMS-790052
were observed with two replicons carrying either the first 100 amino acids of NS5A or the entire NS5A from GT-1a/H77 strain. However, when a Q30H resistant mutation was introduced, the EC$_{50}$ of BMS-790052 against the replicon carrying the entire GT-1a/H77 NS5A was 7-fold higher than that of the replicon carrying only the first 100 amino acids of GT-1a/H77 NS5A. Similarly, in GT1a/J1 strain with a Q30H polymorphism in its NS5A, the EC$_{50}$ of BMS-790052 against the replicon carrying the entire GT-1a/J1 NS5A was 8-fold higher than that of the replicon carrying only the first 100 amino acids of GT-1a/J1 NS5A. Comparisons between GT-1a/H77 and GT-2a/JFH-1 non-chimeric and chimeric replicons also revealed significantly higher EC$_{50}$ values of BMS-790052 against non-chimeric replicons. Conclusion: The viral sequences beyond the first 100 amino acids of NS5A also affect the antiviral activity of BMS-790052.

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Effects of the combination of Lactobacillus rhamnosus and amantadine on influenza A virus infection in mice

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The extensive world-wide morbidity and mortality caused by influenza A viruses highlights the need for new insights into the host immune response and novel treatment approaches. More potent antiviral therapy can be achieved by using drugs in combination. But the kinds of studies have been limited based upon the number of active antiviral compounds that are available. In previous studies, Lactobacilli were found to be effective in the prevention or treatment of influenza virus infection in mice by its immunopotentiating activity. The purpose of the present study was to evaluate whether the combination of Lactobacillus rhamnosus (L. rhamnosus) with amantadine is more beneficial than monotherapy against influenza virus infection in mice. Mice were administrated orally and sublingually with L. rhamnosus by for 10 days, respectively. Subsequently, mice were inoculated intranasally with influenza virus A/NWS/33 (H1N1) strain. Amantadine was given twice a day for 5 days, starting 4h before infection. All mice were observed daily for 14 days. In a mouse challenge study, the combination of L. rhamnosus with amantadine produced improvements in survival and in body weight against lethal challenge with influenza virus. Combination treatments may be necessary due to widespread emergence of drug-resistant viruses. Because of the high frequency of amantadine-resistant viruses in nature, this may limit the use of the compounds as a combination agent. In support of this statement, treatment of amantadine-resistant virus infections with amantadine plus oseltamivir or ribavirin provided no improvement compared to using oseltamivir or ribavirin alone. In this study, the combination of L. rhamnosus with amantadine can provided improved protection against influenza virus infection in mice by enhancing respiratory cell-mediated immune responses. As L. rhamnosus advances through clinical development, it may become a viable option for the treatment of seasonal influenza as well as the next pandemic influenza, either used alone or in combination with amantadine.

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Evaluation of the effects of bioflavonoids on dengue virus type-2 replication

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Bioflavonoids are plant-derived polyphenolic compounds with many health benefits. In the present study antiviral activity of four types of bioflavonoids were evaluated against dengue virus type-2 (DENV-2) in monkey kidney Vero cell line. Anti-dengue activity of these compounds were determined at different stages of DENV-2 infection and replication cycle. DENV replication was measured by Foci Forming Unit Reduction Assay (FFURA) besides quantitative RT-PCR. It was demonstrated that the IC50 of quercetin is 35.7 μg/ml when it was used after adsorption of DENV-2 to the cells. The IC50 of quercetin decreased to 28.9 μg/ml when the cells were treated continuously for 5 hours before virus infection and up to 4 days post infection. The SI values for quercetin were 7.07 and 8.74 μg/ml, respectively. Naringin only exhibited anti-adsorption effect against DENV-2 with IC 50= 168.2 μg/ml and its related SI was 1.3. Daidzein showed a weak anti-dengue activity with IC50=142.6 μg/ml when the DENV-2 infected cells were treated after virus adsorption. The SI value for this compound was 1.03. Hesperetin did not exhibit any antiviral activity against DENV-2. The present study showed specific infection and replication processes targeted by various bioflavonoids. Among all tested bioflavonoids, quercetin demonstrated significant anti-DENV potentials and it should be considered for the development of new and more effective derivatives as anti-dengue therapeutics.

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Effects of fisetin and naringenin against dengue virus in vitro replication
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In vitro antiviral activities of fisetin and naringenin two types of bioflavonoids against DENV-2 (NGC strain) were evaluated. Inhibitory effects of each compound at the different stages of DENV-2 infection were examined using foci forming unit reduction assay (FFURA) and quantitative real-time polymerase chain amplification (qRT-PCR). Fisetin and naringenin showed cytotoxic effects against Vero cells with 50% cytotoxicity (CC50) values of 247, >1000 μg/ml, respectively. Fisetin when added to Vero cells after virus adsorption inhibited DENV replication with a half maximal inhibition concentration (IC50) value of 55 μg/ml and selectivity index (SI) of 4.49. The IC50 value of fisetin was 43.12 μg/ml with SI=5.72 when Vero cells were treated for 5 h before virus infection and continuously up to 4 days post-infection. There was no direct virucidal activity or prophylactic activity of fisetin against DENV-2. Naringenin did not inhibit DENV-2 intracellular replication in Vero cells. Naringenin however, exhibited direct virucidal activity against DENV-2 with IC50 = 52.64 μg/ml but the SI was <1. The present study suggests that among the flavonoids examined, only fisetin showed significant in vitro anti-dengue virus replication activity.

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Discovery of imidazopyridinylthioacetanilide derivatives as potent HIV-1 inhibitors by scaffold hopping
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In continuation of our efforts toward the discovery of potent HIV-1 NNRTIs with novel structures, we have employed a scaffold hopping strategy to explore the chemical diversity space of bioactive compounds. The original arylazolylthioacetanilide platform was replaced with different imidazopyridinylthioacetanilide scaffolds to append the optimal pharmacophore moieties in order to generate novel NNRTIs with desirable potency. The results
showed that some of the new compounds proved to be able to inhibit HIV-1 replication in the low micromolar range. In particular, compound 5b16 displayed the most potent anti-HIV-1 activity (EC50 = 0.214 ± 0.059 µM), inhibiting HIV-1 IIIB replication in MT-4 cells more effectively than DDC (EC50 = 1.394 ± 0.0502 µM) and similarly with NVP (EC50 = 0.200 ± 0.105 µM). The preliminary structure-activity relationships (SARs) of the newly synthesized congeners are discussed, and molecular modeling study is performed to rationalize the SAR conclusions.

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Poster Session 2: Herpes Viruses, Pox Viruses, Other Antiviral Agents and Medicinal Chemistry

Chairs:
4:00 - 6:00 pm
Royton Hall CD, 3rd Floor

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NAOMI: a molecular modelling tool for the prediction of nucleoside analogues activation
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Nucleoside analogues are one of the most important class of antiviral agents, being the cornerstone of several antiviral therapies. The design and identification of novel compounds of this class is an incredibly active research field, with several new structures currently being evaluated clinically (e.g. for the treatment of HCV infections). However, nucleoside analogues are not generally active in the structural form administered to patients and they require in vivo activation to their phosphorylated form before they can acquire their antiviral effect. This activation pathway involves different enzymatic steps, making difficult for researchers to determine a solid Structure-Activity relationships for novel nucleoside analogues. Interestingly, the majority of the proteins involved in this process has been well characterised and their 3D structure is available. Starting from this observation, we decided to work toward the development of an in silico tool that would be able to simulate the activation and metabolism pathways of novel nucleoside analogues. Such model would be extremely useful not only in predicting if a new compound will be correctly phosphorylated, but it would also be useful in identifying at what stage a new structure would fail. Our system (NAOMI) currently includes >20 enzymes, including kinases, deaminases and polymerases, and in this presentation we will discuss our initial, yet very promising, results obtained from its use. We will also discuss the potential for expansion of this model to include viral targets, making also possible for NAOMI to assess the inhibition of the novel nucleoside analogues against such targets.
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Prodrugs of Neuraminidase Inhibitors with High Oral Bioavailability

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We sought to improve the oral bioavailability of neuraminidase inhibitors, a known class of compounds that have poor bioavailability due to their highly polar nature, in order to develop an oral formulation of the drug. We hypothesized that the poor bioavailability could be overcome by targeting the dipeptide transporter, hpept1 or SLC15A1, which is a prevalent nutrient transporter in the intestine. Using a series of model compounds, we found that a major determinant of successful uptake was the stability of the prodrg, which, in turn, was dependent on the physical properties of the linker group of the promoiety group. We tested a series of prodrugs for intestinal drug uptake using standard in vitro uptake/transport assays (Caco-2 and hpept1 overexpressing cell lines), in situ intestinal perfusion permeability studies and standard PK preclinical studies. The final lead series of neuraminidase inhibitor prodrugs that we tested were up to 50 % absorbed under fed and fasted conditions in mice. Importantly, these neuraminidase inhibitors are effective against neuraminidase isolated from oseltamivir resistant influenza virus. These compounds were shown to be effective in lethal challenge studies in mice against influenza A/NWS/33. One of the NI prodrugs was found to be 10 times more effective than the current marketed neuraminidase inhibitor – oseltamivir. In summary, we have developed a prodrug strategy for improving the oral absorption of two potent neuraminidase inhibitors. The prodrugs were shown to be approximately 50% bioavailable and were as good as or better than oseltamivir in influenza challenge models.

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Elucidation of the antitherpetic activity of tenofovir, an anti-HIV selective drug

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The HIV reverse transcriptase inhibitor tenofovir ((R)PMPA) has been shown in the CAPRISA-004 clinical study to prevent HIV sexual transmission by 39% but, surprisingly, also herpes simplex virus type 2 (HSV-2) transmission by 51% (1). We now demonstrated that tenofovir has a direct anti-herpetic activity in a variety of antiviral assays (2). Tenofovir inhibits a broad variety of HSV clinical isolates in human embryonic lung (HEL) fibroblasts and primary human keratinocytes (PHK), primary macrophages, organotypic epithelial 3D rafts and human lymphoid and cervicovaginal tissues ex vivo. Tenofovir also delays HSV-induced lesions and death in topically treated HSV-infected mice. The active tenofovir-diphosphate metabolite has been formed in relevant cell types and inhibits both HIV reverse transcriptase and HSV DNA polymerase. It was concluded that tenofovir solely inhibits HSV infection in vivo upon topical drug administration that allow the generation of local drug concentrations that are considerably higher than achieved by oral application. Thus, a drug that is endowed with a rather marginal antiviral (i.e. anti-herpetic) activity (at an EC50 of ~ 100-200 µg/ml in HEL and PHK cell cultures) may afford a clinically relevant antiviral activity if local drug concentrations can be achieved that exceed its (relatively poor) antiviral activity observed in cell culture. References: (1) Abdool Karim et al. Science 2010329:1168-1174. (2) Andrei et al. Cell Host Microbe 201110:379-389
The utility of plethysmography for measuring lung function in mice infected with HPAIV for use in antiviral or vaccine studies

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Whole-body plethysmography measures impeded respiration in conscious unrestrained subjects. It assesses pressure changes induced by the respiratory movements of an unrestrained animal. It can provide a record of inspiratory time (Ti), expiratory time (Te), relaxation time (Tr), tidal volume (Tv), minute ventilation (MV), peak inspiratory flow (PIF), peak expiratory flow (PEF), and respiratory rate (RR). Enhanced pause (Penh), an index of airway hyper-reactivity and an indicator of changes in airway resistance can also be calculated. Plethysmographic evaluation of mice infected with highly pathogenic avian influenza A H5N1 (HPAIV) showed that almost all parameters measured correlated (Pearson correlation coefficients [PCC] = 0.85-0.98) well with mortality of mice. Mortality of HPAIV-infected mice treated with an efficacious dose of 2'-fluorodeoxycytidine (2'-FdC) (60 mg/kg/d, bid X 8, survivors = 80%) correlated well with Penh values, Te, and Tv (0.84-1.0). Using a less efficacious dose of 2'-FdC (5mg/kg/d, bid X 8, survivors = 50%), mouse mortality correlated well with Penh and measurements of Tv, MV, Ti, PIF, and respiratory rate (PCC = 0.62-0.84). The Penh value calculated for each group of mice was the parameter that was most closely associated with mortality of mice in each group (PCC = 0.71-0.97) a greatly increased Penh value measured 1-2 days before death was often indicative of imminent mortality. Penh values of infected mice treated with the efficacious dose of 2'FdC were much lower in surviving mice than in mice that did not survive the infection, the Penh values equaling the values of uninfected mice. Mice treated with the less efficacious dose of 2'FdC that survived infection had lower Penh values compared to those mice that died in that treatment group. However, no significant correlations with mortality and saturated blood oxygen values were observed in the HPAIV mouse model used in this study. Thus, plethysmography appears to be a valid way to measure lung function during an HPAIV infection of mice and to measure the effects of efficacious and non-eficacious doses of drug or vaccine on lung function in HPAIV-infected mice.

Design, synthesis and evaluation of novel anti-CHIKV compounds.

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Chikungunya virus (CHIKV) is an emerging, mosquito-borne pathogen responsible for major outbreaks of febrile arthralgia in humans. Currently, neither a vaccine nor an effective antiviral drug is available for the treatment of this infection. The single-stranded, positive-sense RNA genome encodes four non-structural proteins (nsPs), which represent promising targets for antiviral drug development. Of particular interest is the viral nsP2 protein, a cysteine-protease essential for proteolytic processing of the non-structural polyprotein into the four mature nsPs. A homology model of the CHIKV nsP2 protein, which we developed for a previous virtual screening study, allowed us to identify one compound, which prevented virus-induced cell death at low µM concentration. Preliminary optimisation studies with this first hit, using a series of molecular dynamics simulations on nsP2/natural substrate and nsP2/hit complexes, allowed rational selection of novel analogues, which were evaluated for selective antiviral activity in a CHIKV-cell-based assay. Several were found to inhibit virus-induced cytopathic effect at low µM concentration as well. Based on this first SAR exploration, we designed and synthesized a series of new derivatives
to further optimise the antiviral activity of this compound class. In this presentation, we will discuss the rationale of this project, the chemical approach, and the biological results obtained with this series of promising anti-CHIKV agents.

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Molecular Modelling Studies on the VPg-Polymerase Complex of Enteroviruses.

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Picornaviruses are small, non-enveloped, (+) RNA viruses involved in several human and animal diseases. Among human pathogens, Enteroviruses, a genus including coxsackie-, echo- and polioviruses, cause meningitis, encephalitis, carditis and poliomyelitis. Moreover, enteroviruses are suspected to have a role in the pathogenesis of insulin-dependent diabetes mellitus. There is no specific treatment for picornaviral infections and there is a need for new therapeutic agents. One of the most promising targets for antiviral drug design is the RNA replication, a process catalyzed by the RNA-dependent RNA polymerase, 3Dpol. The initial role of the RdRP is the covalent attachment of two UMP molecules to the hydroxyl group of a tyrosine residue located in the small viral protein VPg. The product, Vpg-pUpU, then serves as primer for production of full length RNA. Since the VPg uridylation is the first step of the RNA replication, it is possible that a small molecule could selectively inhibit this process. To investigate this hypothesis, we have performed a series of molecular dynamics simulations (MD) to analyze the interactions between the enzyme and primer for both poliovirus and coxsackievirus. To date, no structure of the poliovirus and coxsackievirus VPg-pUpU-3D polymerase complex has been solved, thus our initial models were created using the structure of FMDV-3D-Vpg complex as a template. In particular, we have studied the area where the last six residues of VPg are placed in the RdRP complex. To validate the importance of the putative VPg–polymerase contacts, in silico aminoacid mutations for individual residues of VPg have been performed and the resulting VPg–polymerase complexes were simulated using MD. The results obtained from these calculations could be useful in the design of novel compounds that inhibit this specific biological process.

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Design, Synthesis and Biological Evaluation of Piperidine Substituted Triazine Derivatives as Potent HIV-1 NNRTIs

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Diarylpyrimidine (DAPY) derivatives, one family of HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) with excellent potency against wild-type and resistant mutant strains of HIV-1, have attracted considerable attention over the past few years. Recently, in order to improve pharmacokinetic profiles of DAPYs, a series of piperidine linked aminopyrimidine derivatives was reported with good potency in both enzyme and antiviral assays. In continuation of our efforts toward the discovery of potent HIV-1 NNRTIs with novel structures, “follow-on”-based drug discovery strategies were applied to the chemical evolution of these compounds. In the present investigation, we have synthesized a novel series of piperidine substituted triazine derivatives, and evaluated for their antiviral activity against of HIV-1 and HIV-2 in MT-4 cells, as well as HIV-1 RT inhibitory activity. Most
compounds displayed promising activities against wild-type HIV-1 with EC_{50} values in low nanomole concentration, better than those of Nevirapine, Delavirdine, Zidovudine and Dideoxycytidine, and higher potency towards the resistant mutant strain K103N/Y181C than those of Nevirapine and Delavirdine. Further studies are ongoing in our laboratories and will be reported in due course.

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Cyclic forms of selected acyclic nucleoside phosphonates are particularly less active than their parent counterparts against Epstein-Barr virus replication in P3HR-1 cells, but not in Akata cells

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Introduction: We evaluated the inhibitory effects of selected acyclic nucleoside phosphonates (ANPs), including HPMPC, HPMPA, HPMP-5-azaC, 3-deaza-HPMPA, HPMPDAP, and their respective cyclic forms, in vitro against Epstein-Barr (EBV) replication in P3HR-1 and Akata cells. Based on striking differences observed in the antiviral activities between cyclic and non-cyclic forms of ANPs, we further investigated the potential involvement of a cellular CMP phosphodiesterase (CNPase; EC 3.1.4.37). CNPase is known to hydrolyze cAMP and cCMP, and was also shown to convert cyclic-HPMPC into HPMPC. Methods: The EBV lytic cycle was induced in P3HR-1 and Akata cells, and cultures were further incubated in the presence of different concentrations of ANPs. After 5 days of treatment, concentrations reducing viral DNA loads by 50% (IC_{50}) were determined by real-time quantitative PCR. Western blot was performed to identify CNPase expression level in activated and latently infected P3HR-1 and Akata cells. CNPase cDNA was sequenced to determine potential mutations. Results: In P3HR-1 cells, all cyclic derivatives of ANPs, with the exception of cyclic HPMP-5-azaC, showed IC_{50} values 10- to 40-fold higher than those of their non-cyclic counterparts. In contrast, in Akata cells the IC_{50}'s of cyclic and non-cyclic forms of ANPs were comparable. Hence, the role of CNPase was investigated to explain these differences in activity. CNPase was found to be expressed at comparable levels in both cell lines. Sequencing of P3HR-1 CNPase cDNA showed Q207R amino acid substitution, while in Akata cells no amino acid changes were found. Discussion: Our studies showed the potent anti-EBV activities of various ANPs in vitro, highlighting differences between two EBV-positive cell lines. We hypothesize that the differential inhibitory effects between cyclic and non-cyclic forms of selected ANP in P3HR-1 cells is due to impaired CNPase activity. While the Q207R mutation is described as a natural variant of CNPase, its potential effect on the metabolism of ANPs remains unknown. Therefore, experiments are ongoing to examine the metabolism of cyclic HPMPC in P3HR-1 and Akata cells.

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Varicella-zoster Virus Resistance to L-BHDU, a Dioxolane L-Nucleoside, is Dependent on Thymidine Kinase
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Current treatments for the α-herpesvirus varicella-zoster virus (VZV) are acyclovir (ACV) and penciclovir and their prodrugs valacyclovir and famciclovir. Alternatives are phosphonoformate (PFA), and brivudin (BVDU, Europe only). Additional antiviral drugs with increased potency are needed, especially for resistant viruses and to treat post-herpetic neuralgia. We found that the bromovinyl uracil nucleoside L-BHDU and its valyl derivative were effective against VZV in culture and in a mouse model at lower doses than ACV. In VZV-infected cells, only L-BHDU monophosphate was detected, indicating that it may have a novel mechanism of action compared to ACV, which causes DNA chain termination as ACV triphosphate. To begin the study of L-BHDU mechanism, resistant strains were isolated and analyzed. The parent virus, VZV-BAC-Luc, is a recombinant clinical isolate that expresses eGFP
and firefly luciferase, enabling measurements of virus spread by bioluminescence imaging. VZV-BAC-Luc strains resistant to L-BHDU (8), ACV (8), BVDU (6), or PFA (in progress) were isolated and the DNA was extracted for sequencing. Remarkably, the thymidine kinase (TK) gene (ORF 36) from all isolates had the identical mutation (G to A) at position 63, causing an amino acid change from glycine to arginine (G22R) in the ATP-binding domain. We are investigating whether the parent virus carries this mutation at a low frequency. Resistance to EC90 concentrations (L-BHDU and BVDU, 2µM; ACV, 20 µM) was reached in 7-8 passages over 45-50 days. The growth kinetics of the resistant strains were normal. Two of the L-BHDU-resistant strains were evaluated for cross-resistance to the other drugs, and, as expected, they were resistant to ACV and BVDU but not PFA. Similarly, two ACV-resistant strains were also resistant to L-BHDU. These results confirm that L-BHDU activity is dependent on TK. Experiments are underway to elucidate the targets of L-BHDU-MP in VZV-infected cells.

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The picornavirus inhibitor enviroxime inhibits HCV RNA replication in vitro by inhibiting PI4KIII.
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Enviroxime [2-amino-1-(isopropylsulfonyl)-6-benimidazole phenyl ketone oxime] is since long known as an inhibitor of the in vitro replication of rhinoviruses and enteroviruses. Picornaviruses resistant to enviroxime carry mutations in viral protein 3A. It was previously shown that enviroxime inhibits RNA replication of picornaviruses by inhibiting phosphatidylinositol 4-kinase IIIβ (PI4KIIIβ). PI4KIIIβ regulates the synthesis of enteroviral RNA by creating a micro-environment of phosphatidylinositol 4-phosphate (PI4P) lipids at the viral replication complexes. Recently, PI4KIIIα was identified as an important host factor for HCV replication. Knockdown of PI4KIIIα expression drastically changed the membranous web morphology. We here report that enviroxime inhibits HCV genotype 1a and 1b subgenomic replicon replication. Viral RNA replication is inhibited in a dose-dependent manner with an EC50 value of 0.26 ± 0.06 µM (CC50=28 µM). Following 8 weeks of culturing, enviroxime-resistant replicons were generated (EC50=19 µM). Genotyping of the resistant replicon population revealed the presence of two mutations in NS4B (V38M, D167E) and several in NS5A. Interestingly, both HCV NS4B and picornavirus 3A are membrane associated proteins that are involved in the formation of the membranous web. Introduction of the V38M mutation or swapping of NS5A of the enviroxime-res replicon into a wild-type genome did not result in a transfer of the resistant phenotype. Replicon carrying the D167E mutation had a pronounced reduction in replication fitness as compared to wild-type and could therefore not be assayed for susceptibility to enviroxime. siRNA knockdown of PI4KIIIα in the enviroxime-res replicon containing cells is currently being performed to confirm the correlation between the anti-HCV activity of enviroxime and inhibition of PI4KIIIα. Furthermore, PI4P stainings will provide more information on the inhibition of PI4P synthesis upon enviroxime treatment. In summary, we here report on the in vitro anti-HCV activity of enviroxime. Genotyping of the enviroxime-res replicon revealed the presence of mutations in NS4B and in NS5A. The observation that HCV replicons can adopt a strategy to replicate (largely) independently from an essential host factor is intriguing.

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Targeting HIV gp120: Design, Synthesis and Biological Evaluation of Novel Polyboronate Carbohydrate Binding Agents
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The HIV envelope protein gp120 plays a fundamental role in the viral infection and in the immune response. It contains an unusually high amount of clustered terminal mannose residues, which are rarely present in mammalian cells, and thus represents a potential target for the development of new antiviral agents. It has been reported that carbohydrate-binding agents (CBAs) are able to block viral entry by interacting with the cis-diol moiety present in the terminal glycans of the viral envelope, impeding the interaction with the host cells. Phenyl boronic acids have shown the same ability to interact with cis-diol present in carbohydrate (i.e. mannose) structures. A plethora of mono-phenyl boronic and bis-phenyl boronic acids has been investigated for their ability to inhibit the HIV replication. Unfortunately, neither anti-HIV activity nor HIV-1 gp120 binding has been detected. These compounds displayed low, if any cytotoxicity. These results prompted us to further investigate this type of compounds as potential anti-HIV agents. We report herein, the design, synthesis and biological (i.e. anti-HIV) evaluation of larger polyboronate aggregates which contain a much higher number of phenylboronic acid units.

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CYSTUS052, a polyphenol rich plant extract, exerts potent anti-influenza activity by preventing viral attachment to host cells in a non-pharmacological manner

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Infections with Influenza A viruses are still a serious burden for mankind. Although the recent influenza pandemic in 2009 took a rather mild course, the relatively long time period since a vaccine was available highlighted the fact, that vaccination is not an option for the early phase of a pandemic outbreak. In addition, the increasing incidence of resistance to the currently licenced influenza drugs indicates the urgent need for novel antivirals against the flu. Antiviral acting plant products from traditional medicine could be a promising alternative for prophylaxis and therapy of influenza. Here we demonstrate that the polyphenol rich plant extract CYSTUS052 from the Mediterranean plant cistus incanus exerts a potent anti-influenza virus activity in cells and animals infected with various influenza viruses including those of the H5N1 and H1N1v type. Interestingly, viruses did not develop resistance to CYSTUS052 upon consecutive passaging. CYSTUS052 did not exhibit apparent harming effects on cell viability and did not influence metabolism or proliferation. Thus, surprisingly host cells appeared to be inert to the action of CYSTUS052 since the compound could neither induce nor block cell activation by extracellular ligands in a variety of ligand/receptor/signaling systems analysed. Mechanistically, the protective effect appears to be due to a physicochemical and reversible binding of the CYSTUS052-ingredients to the virus surface, preventing virus-binding to cellular receptors. Thus, the extract is not only an efficient antiviral against influenza viruses in vitro and in vivo but also acts via a novel mechanism, namely non-pharmacological interaction with the virus particle to prevent entry into host cells. Since these plant extracts are already in use in traditional medicine for centuries without reports of side effects, local application of CYSTUS052 to the respiratory tract may be a promising approach for prophylaxis and therapy against influenza.

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Chemical Genomics Profiling of Host Kinase Inhibitors as Broad Spectrum Antivirals.

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Human host kinases are of therapeutic interest since they are used for replication by multiple viruses, and therefore represent antiviral targets. In this study, we have used high-throughput screening (HTS) and a kinase-targeted small molecule library (26,878 compounds) to identify new classes of antivirals in an in vitro influenza/human cell infection model. The kinase-targeted library was screened against H1N1, H5N1, and H3N2 strains of influenza. Active antiviral compounds were identified and their potential kinase targets were biochemically investigated using global kinase activity profiling. Our goal was to identify compounds that reduce viral replication against one or all three tested strains of influenza by inhibiting a host kinase that is critically-important for the viral life cycle. Forty of the top hits from our influenza HTS were evaluated by 8-point concentration response assays for efficacy and toxicity against all three strains. Using these methods, we confirmed that hits from the kinase-targeted library are very effective antivirals (EC50s ranging from 85 nM to 48 μM, with selective indices from 4 to >500) with potency and selectivity indices that rival currently-approved antiviral drugs. A set of nucleoside-analog scaffolds were identified that were effective against one or more strains of influenza. We used kinase activity profiling to identify the putative target(s) of two of the compounds that were effective against H1N1 and H5N1 (IC50s of 85 and 565 nM), but not H3N2 influenza strains. Activity profiling of these compounds against 268 human kinases indicated that both structurally-similar compounds inhibited phosphatidylinositol-4-phosphate 3-kinase C2 by 46 and 31%, respectively, in a cellular lysate. PI3K2CB has been implicated in viral entry processes, and does not currently have documented inhibitors. Our study demonstrates three facts: 1) The targeted kinase inhibitor library contains numerous compounds with confirmed anti-influenza activity; 2) Many of these active compounds are specific for a single strain of influenza, and 3) Several compounds possessed broader activity against multiple influenza strains, suggesting that they may also be effective against other RNA virus families.

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Synthesis of 4'-ethynyl-2'-deoxy-4'-thioribonucleosides and discovery of a highly potent and less toxic NRTI

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We have recently reported synthesis and anti-HIV activity of 4'-substituted 4'-thiothymidines. Among these novel nucleoside analogues, 4'-ethynyl-4'-thiothymidine 1 showed more potent activity than that of the respective thymidine derivative 2. These findings stimulated us to synthesize the respective cytosine-, adenine-, and guanine analogues. In this conference, the synthesis and anti-HIV-1 activity of the title compounds 3-5 will be presented.

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Synthesis of 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives with potential anti-HIV activity

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Background: Nine novel uracil analogues (1a-e, 2a-d) were synthesized and evaluated as inhibitors of human immunodeficiency virus type-1 (HIV-1). Methods: Key structural modifications included introduction of other functional groups into 6-position of 1-benzyl-6-chloro-3-(3,5-dimethylbenzyl)uracil or N1-alkylation of 3-(3,5-dimethylbenzyl)-5-fluorouracil. Results: These compounds showed only micromolar potency against HIV-1 in MT-4, though two of them 6-azido-1-benzyl-3-(3,5-dimethylbenzyl) uracil (1d) and its 6-aminopropyl analog (1e) were highly potent (half maximal effective concentration (EC50) = 0.067 and 0.069 mM) and selective (selectivity index (SI) = 685 and 661), respectively. It is suggested that 1d converted to 1e before exerting its anti-HIV activity. Conclusions: We discovered two 6-substituted uracil derivatives (1d and 1e) as novel anti-HIV agents [1]. These compounds should be further pursued for their toxicity and pharmacokinetics in vivo as well as antiviral activity against NNRTI-resistant strains. Reference: [1]: Isono Y, Sakakibara N, Hamasaki M, Ikejiri M, Maruyama T. Synthesis of 1-benzyl-3-(3,5-dimethylbenzyl)uracil derivatives with potential anti-HIV activity. Antiviral Chemistry & Chemotherapy 2011 22: 57-65.

Fig. Antiviral activity of 1,3-disubstituted uracils against HIV-1.

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Neonatal Herpes Caused by an Acyclovir-Resistant Herpes Simplex Virus Type 1

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[Introduction] Administration of acyclovir (ACV) is the standard therapy for herpes simplex virus type 1 (HSV-1) infections. We report here a neonatal central nervous system (CNS) HSV-1 infection intractable to ACV-administration. The viral load in the cerebrospinal fluid (CSF) decreased by the initial treatment with ACV but increased again after a few weeks. Vidarabine was then added, resulting in remission. The objective of this study was to virologically assess whether ACV-resistant (ACV) HSV-1 emerged in this patient, as was suggested by the clinical course.

[Materials and methods] CSF samples were collected twice, first on admission and second just before the addition of vidarabine and tested for the viral thymidine kinase (vTK) gene amplification by nested PCR. The nucleotide sequences were determined and the mammalian cell expression vectors for the vTK were constructed using pTargetT vectors (Promega). The sensitivity of the causative virus to ACV was assessed by measuring inhibitory effect of ACV on replication of vTK-deficient HSV-1 in 293T cells transfected with these vectors. [Results] A nucleotide mutation (G375T) leading to a single amino acid substitution (Q125H) in the vTK polypeptide was demonstrated in the second sample. The inhibitory effect of ACV on replication of vTK deficient HSV-1 was significantly weaker in 293T cells transfected with pTargetT-vTK(375T), containing the G375T mutation in the vTK gene, than in cells treated with pTargetT-vTK(375G) containing no mutation. [Discussion] These results indicate HSV-1 with the G375T mutation in the vTK gene is resistant to ACV. This is the first patient report of a virologically proven neonatal ACV HSV-1 CNS infection. The assay system used here can be applied in evaluating the drug
resistance of HSV associated with vTK mutation when full-length vTK gene is available. [Contributors] Kazumi Yamaguchi-Kinoshita, Mutsuyo Ito-Takayama, Chang-Kweng Lim (National Institute of Infectious Diseases), Professor Takashi Igarashi (Department of Pediatrics, the University of Tokyo)

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Creation of Universal Vectors for Prophylactic and/or Therapeutic Recombinant Virus Vaccines

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Vaccination against infectious agents has proven to be the best way to prevent infectious diseases. We have created genetically modified recombinant M gene mutant of the Indiana serotype of vesicular stomatitis virus (VSVind) and M gene mutant of the New Jersey serotype of VSV (VSVNJ) as universal vectors for the development of recombinant virus vaccines. The priming vaccine vector should be antigenically distinct from the boost vaccine vector in order to maximize the boost effects. rVSVind with the mutations of G21E/M51R/L111F in the M protein (VSVindGML) and rVSVNJ with the mutations of G22E/M48R+M51R in the M protein (rVSVNJGMM) was attenuated to a degree that mice injected with 50 million of these genetically modified infectious viruses directly into the brain showed no neurological signs or any other adverse effects. In contrast, 100 infectious wild-type VSVind or wild-type VSVNJ kills mouse within 48 hours. Foreign genes inserted into these VSV vectors elicit strong B cell and T cell immune responses when we prime animals with VSVind(GML) followed by boost immunization with rVSVNJ(GMM) carrying the same genes of interest. Animals can tolerate more than 5 x 10^9 PFU each of recombinant infectious VSVind(GML) and recombinant infectious rVSVNJ(GMM) and showed high levels of gene expression and immune responses. Our results show clearly that rVSVind(GML) priming and rVSVNJ(GMM) boosting is the best way to induce ultimate humoral and cellular immune responses. We will describe the advantages of these dual serotype VSV vectors for future vaccine development against infectious diseases and cancers.

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Microwave assisted synthesis and antiviral activity of some Mannich & Schiff bases of 2-oxyindole derivatives

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A series of microwave assisted Mannich Schiff bases of 2-oxindole have been prepared by reacting 2,3-dioxindole / Mannich bases of 2,3-dioxindoles with thiosemicarbazides in good yields. Structures of all these derivatives were established by IR, ¹H-NMR and mass spectroscopy. All compounds were tested for cytotoxicity & antiviral activity against a broad variety of viruses. Derivatives A34, 35, 38, 43, 44 and A62 showed activity against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G) and vaccinia virus, in the range of 9-20 μM. We also observed rather high cytostatic, but lower cytotoxic activities for these compounds. Therefore, it cannot be excluded that the observed antiviral activities observed in monolayer cell cultures at subtoxic concentrations are due to underlying antimetabolic activity of the compounds.

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murine norovirus-1 RNA-dependent RNA polymerase in thiouridine or ribavirin

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Crystal structures of complex with 2-

Kyung Hyun Kim¹, ²Korea University, Seoul,
Murine norovirus-1 (MNV-1) shares many features with human norovirus (HuNoV) and both are classified within the norovirus genus of *Caliciviridae* family. MNV-1 is used as the surrogate for HuNoV research since it is the only form that can be grown in cell culture. HuNoV and MNV-1 RNA dependent RNA polymerase (RdRp) proteins with the sequence identity of 59% show essentially identical conformations. Here we report the first structural evidence of 2-thiouridine (2TU) or ribavirin binding to MNV-1 RdRp, based on the crystal structures determined at 2.2 Å and 2.5 Å resolutions, respectively. Cellular and biochemical studies revealed stronger inhibitory effect of 2TU on the replication of MNV-1 in RAW 264.7 cells, compared to that of ribavirin. Our complex structures highlight the key interactions involved in recognition of the nucleoside analogs which block the active site of the viral RNA polymerase.

![Image](image_url)

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**The Activity of New Cage Compounds Against Influenza Viruses.**


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Treatment and prophylaxes of high pathogenic influenza virus H5N1 and related types of viruses requires development of more performance drugs. It’s well known that many of adamantane derivatives have antiviral properties and used for treatment of influenza. The presence of great number of high active compounds indicates some common principles of antiviral action of compounds, containing saturated cage moiety. During our investigation we have prepared a number of derivatives of adamantane: amides, amino, oximino, nitroso derivatives and wide range of adamantyl substituted oxygen and nitrogen containing heterocycles. Antiviral activity of synthesized compounds was evaluated against influenza viruses A H5N1, H7N1, H1N1, H3N2 on cell cultures fibroblastes of chicken embryos, MDCK, RL-33, Hep-2C. Among the prepared compounds remarkable activity against H5N1 virus has been shown by adamantane derivatives with the both amino and oximino groups in the side chain. Similar results were obtained for aminopyrazole derivative of adamantane against H1N1 virus. Some of chloronitroso derivatives of adamantyl substituted olefins were more potent against both H5N1 and H7N1 viruses and ethylenediamine derivative has shown approximately equal activity against all studied viruses.

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**The Activity of the New Adamantane Derivatives Against the Orthopoxviruses**

At present time population becomes vulnerable to orthopoxvirus infections since the discontinuation of regular vaccination. So the problem of development of drugs for treatment of orthopoxviruses becomes actual. Functional derivatives of cage compounds are as is known one of perspective substances for development of antiviral agents. Wide range of adamantane derivatives has been prepared and antiviral potency of synthesized compounds was evaluated against following orthopoxviruses: vaccinia, cowpox, mousepox and monkeypox in cell cultures (Vero, MK-2). More than ten compounds have very good antiviral action and very low acute toxicity. Among them it is necessary to note 2-adamantyl amides, adamantyl and diadamanyl substituted pyrazoles with high activity against vaccinia virus at µM concentration and diadamantyl substituted imidazole has good potency against monkeypox (IC$_{50}$<1 mM). Structures of compounds having activity against poxviruses allow to suppose that their action occurs at the later stages of viral reproduction. Acknowledgements: The work has been carried out with financial support by the Ministry of Education and Science of Russian Federation and using the equipment of Joint Use Center "The study of physicochemical properties of substances and materials".

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**Antiviral Activity of Oversulfated Smaller Molecules of Sulfated Exopolysaccharide from the Marine Microalga *Gyrodinium impudicum* strain KG03**

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The sulfated exopolysaccharide p-KG03, which is produced by the marine microalga *Gyrodinium impudicum* strain KG03, had a molecular weight of 1.87$\times$10$^6$, and was characterized as a homopolysaccharide of galactose with uronic acid (2.96%, w/w) and sulfate groups (10.32% w/w). It exhibited antiviral activity in vitro against several enveloped viruses and also some naked viruses. It generally showed little cytotoxicity (CC$_{50}$, >100 ug/ml) but some to certain immune cells such as MT-4 (human T-lymphocytes) and RAW (mouse macrophage) (CC$_{50}$, <10 ug/ml). Fragmentation and oversulfation were performed with p-KG03. The smaller oversulfated forms showed decreased antiviral activity compared to p-KG03, and much less cytotoxicity to the certain cells (CC$_{50}$, from <10 ug/ml to >100 ug/ml). The biological activities of these compounds may be useful in biotechnological and pharmaceutical products.
The purpose of this study is to research antiviral or virucidal activity of callus extracts (CEs) and to characterize proteolysis inhibitors (PIs) prepared from different plants. Freeze-dried powders obtained from calluses and their spent culture media were reconstituted and clarified by centrifugation. Antiviral or virucidal activities were determined using influenza virus A/Hong Kong/1/68 (H3N2) suspended in culture media in the presence (experiment) and in the absence of CE (control). The number of infectious viral particles in control and experimental samples were determined after incubation by titration on fragments of chorio-allantoic membranes of 12-14-day old chick embryos. All experiments were performed in triplicates. Toxicity of the investigated CEs and PIs in studied doses was tested using the infusorian Colpoda steinii culture. The highest levels of virucidal activity were found in the CEs from Nicotiana suaveolens and Nicotiana alata (3.2 and 2.3 log10 TID50 as compared to control, respectively). Virucidal activities of CEs from Nicotiana exelsior, Nicotiana rustica and Nicotiana trigonophylla were well expressed (1.5 to 1.9 log10 TID50 as compared to control). CE from Nicotiana pauciflora had low activity while CEs from Nicotiana goodspedii, Ficus sur, Physalis philadelphica, and Solanum pseudocapsicum did not demonstrate any virucidal effect. CEs from Physalis philadelphica and Solanum pseudocapsicum that did not demonstrate virucidal activity, inhibited virus reproduction like CEs from Campanula kemulariae, Mammillaria multiceps, and Bauhinia tomentosa. PIs from soybeans, haricot and potatoes inhibited virus reproduction too. All investigated CEs and PIs in studied doses were non-toxic for the Colpoda steinii culture.

Conclusions: CEs from different tobacco-plants have a wide spectrum of anti-influenza virucidal activity in non-toxic doses. PIs and some CEs from plants strongly inhibit influenza virus reproduction. Therefore plants and their calluses are perspective sources for obtaining substances with antiviral and/or virucidal abilities.

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Action mechanisms of tricin on anti-cytomegalovirus effect

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Human cytomegalovirus (HCMV) persists as a lifelong latent infection. However, HCMV is frequently activated in immunocompromised individuals, thereby causing severe morbidity and eventual mortality. Symptomatic HCMV infection has been treated with ganciclovir (GCV), but the appearance of GCV-resistant viruses is a recurrent problem in the treatment of immunocompromised patients with HCMV infection. Although PFA and CDV have been used in combination with GCV for the treatment of GCV-resistant HCMV, these treatments are not always successful. Therefore, effective new anti-HCMV agents and regimens need to be developed. We show that the tricin (4',5,7-trihydroxy-3',5'-dimethoxyflavone), a derivative from Sasa albo-marginata, have anti-HCMV properties in the human embryonic fibroblast cell line MRC-5. Exposure of fibroblasts to tricin inhibited both infectious virus production and replication of HCMV, with concomitant decreases in the levels of both the transcript of the HCMV immediate early (IE) gene and the CXC chemokine IFN-inducible T cell alpha chemotactrant (I-TAC or CXCL11) gene. We also found that the transcripts of HCMV IE and the replication of HCMV were decreased in CXCL11 gene-knockdown cells. These results suggest that tricin is a novel compound with potential anti-HCMV activity and that CXCL11 is one of the chemokine involved in HCMV replication and it is possible that CXCL11 is the one of targets of tricin.
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Antiviral effect of the specific immunoglobulin against HSV-1
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Today the therapy of viral infections includes usage of ethyotropic chemical preparations, immunopreparations and interferons. Herpetic infection leads to the common spectrum of different in severity of clinical course human illnesses. The effectiveness of immunoglobulins in treatment of viral infections has been proven in many scientific and practical studies. On the basis of human immunoglobulin we developed specific standard preparation of immunoglobulin (IG) against Herpes simplex virus type 1(HSV-1). The aim of this work is to determine in vitro ant Hernandez activity of IG against HSV-1. The study was carried out on the AÎE-21 cells culture on the model HSV-1. It is shown that IG preparation on the 100% neutralizes the virus infection in dilutions 1:32 at 45 min. of exposure, and in dilution 1:256 – at 60 min. of incubation. The virus neutralizing activity lower than 30% was observed at dilution of the preparation more than in 1000 times. It is shown that the IG preparation in high concentration (dilution 1:32) totally blocks the virus reproduction in cells regardless from the treatment scheme. Usage of less concentration of the IG preparation gave us ability to find the dependence of antiviral action from the scheme of the preparation insertion. Analysis of results of anti-viral activity of the IG preparation, got at insertion of the preparation 24 hours prior to the cells infecting or after the infecting with the virus, showed that the dependence of the value of inhibition on the concentration of the IG has a linear character. The antiviral activity was discovered in dilutions diapason 1:16 – 1:64. The cells treatment with the IG preparation 1 hour prior to the infecting with the virus did not shown any substantial influence to the reproduction of the virus at the usage of the preparation dilutions lower than 1:32. At the medical treatment scheme, i.e. the IG preparation insertion after infecting of the cells with the virus, the preparation was active in dilutions 1:16 – 1:256. At this treatment scheme with the preparation even in dilution 1:1024 we can observe 20% inhibition of viral replication and the addition of the preparation in the titre 1:128 a 50% inhibition was observed.

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DiPPro-Nucleoside Diphosphate Prodrugs of 2',3'-Dideoxyuridine (ddU) and 2',3'-Dideoxy-2',3'-didehydrouridine (d4U)
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Despite their close structural similarity to 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) the uridine analogs 2',3'-dideoxy-2',3'-didehydrothymidine (d4T) and 2',3'-dideoxyuridine (ddU) show no activity against HIV in their nucleosidic form. However, the corresponding nucleoside triphosphates proved to be efficient inhibitors of the HIV reverse transcriptase.1 Assuming, that the first phosphorylation step of the intracellular metabolism to the nucleoside triphosphate is the rate limiting step, d4U and ddU were subject to several nucleoside monophosphate prodrug systems in the past. Although the release of the corresponding monophosphates could be observed in many of these studies, this only led to moderate antiviral activity. Due to these disappointing results, ddU and d4U are interesting targets for the nucleoside diphosphate prodrug system (DiPPro) in that two 4-acyloxybenzyl moieties compensate the negative charges of the β-phosphate of a nucleoside diphosphate leaving the α-phosphate unprotected to prevent cleavage of the anhydride bond.2 The masking units are cleaved enzymatically inside the cell and the nucleoside diphosphate can be released. This prodrug approach was successfully used for different nucleoside analogs like 3'-azidothymidine (AZT) or d4T. Here, we report on the synthesis and properties of DiPPro-nucleoside diphosphates of d4U and ddU using masking units with alkyl chains of different length in the
acyl moiety to study the lipophilicity effect of the compounds. Our investigations include the chemical synthesis, the hydrolysis behavior in different media and antiviral testing of the new compounds.


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Broad Spectrum Antiviral Activity of First Generation Methylene cyclopropane Analogs
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Methylene cyclopropane nucleosides have proven to be active against many of the human herpesviruses. The most active compound of this class is cyclopropavir (CPV), which exhibits good antiviral activity against HCMV, EBV, both variants of HHV-6 and human herpesvirus (HHV-8). CPV has two hydroxymethyl groups on the methylene cyclopropane ring, but analogs with a single hydroxymethyl group are also quite active and exhibit a broader spectrum of antiviral that also includes hepatitis B virus and human immunodeficiency virus. Recently, a large set of mono-hydroxymethyl compounds with ether and thioether substituents at the 6-position of the purine was synthesized. Some of these analogs had a broader spectrum of antiviral activity than CPV in that they also inhibited the replication of HSV-1, HSV-2, and VZV. Interestingly, the antiviral activity of these compounds also was less dependent on the activity of the HCMV UL97 kinase than was CPV, and was relatively unaffected by the absence of thymidine kinase activity in HSV. These data indicate that the mechanism of action of these analogs was distinct from that of CPV. They also suggest that they might be useful as a broad spectrum antiviruses agent and should be effective in the treatment of resistant infections.

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Stereoselective Synthesis, Antiviral Activity and Stability of Methyl-Substituted cycloSal-Pronucleotides
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Several nucleoside analogues such d4T, ddC or AZT are used as potent and selective inhibitors of the replication of human immunodeficiency virus. The ultimately active compound of nucleoside analogues is the corresponding 5'-triphosphate which is formed intracellularly by kinases. Often the first phosphorylation to the nucleotide is the rate limiting step due to the high specificity of the involved kinases. To bypass this limitation, several pronucleotide strategies, e.g. the cycloSal-technique, have been developed to mask nucleotides and thus facilitating their transport through the membrane. Some of these pronucleotides are P-chiral compounds and were originally isolated as diastereomeric mixtures only. Recently, we reported on diastereoselective syntheses of cycloSal-phosphate triesters and nucleoside phosphoramidates based on chiral auxiliaries or chiral leaving groups. Using this convergent synthesis we synthesized here different methyl-substituted cycloSal-pronucleotides of d4TMP with very high diastereoselectivities in satisfying chemical yields. The individual diastereomers were tested against HIV-
1 and HIV-2 infected CEM/0 and HIV-2 infected CEM/TK cells. All cycloSal-compounds tested showed significant antiviral activity against both HIV-strains in wild-type CEM/0 cells and also strong activity in the mutant cell line. The antiviral activity depended strongly on the chirality at the phosphate group as well as the substitution pattern of the cycloSal-moïety. In CEM/TK cells the difference in antiviral activity between the individual diastereomers was found to be 7- to 20-fold. In addition, the stability of the individual diastereomers in aqueous phosphate buffer and in CEM/0 cell extracts was studied. In this study, large differences in the half lives of the methyl-substituted cycloSal-compounds were found but correlate with the electronic properties of each substitution pattern. Again, also the stabilities of the individual diastereomers of a cycloSal-triester were found to be different. The results obtained here clearly confirm the importance of the need for a diastereoselective synthesis of the cycloSal-pronucleotides.

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Synthesis of Novel CADA Analog Prodrugs Designed to Act as anti-HIV Agents via Down-Modulation of the CD4 Receptor

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Cyclotriazadisulfonamide (CADA) inhibits HIV replication by specifically down-modulating expression of the CD4 receptor protein on host cells. More than 100 analogs of CADA have been synthesized so far in order to enhance potency, reduce toxicity, and improve physical properties, especially solubility and cell permeability. Current studies are aimed at developing a pro-drug approach involving the novel bis(aminomethylbenzenesulfonyl) CADA analog ES04. According to our 3D-QSAR model, this compound is expected to have a CD4 down-modulation potency similar to that of CADA. ES04 will be the parent compound for different CADA-prodrugs bearing dipeptide chains that are covalently bonded to the two amino groups of the aminomethylbenzenesulfonyl side arms. Cleavage of these chains by dipeptidyl-peptidase IV is expected to convert the prodrugs into ES04. The synthesis of ES04 uses a new palladium-catalyzed macrocyclization method involving the bis(cyanobenzenesulfonyl) analog of CADA. The anti-HIV and CD4 down modulation activities of the novel CADA compounds are presented.

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POSSESSING BROAD ACTIVITIES

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The aim of the work was to study antiviral properties of the purified extract from two grass species Deschampsia caespitosa L. and Calamagrostis epigeios L. wherein the major active substance was identified as glycosylated 3′,7′-dimethylquercetin (DMQ) containing glucose and rhamnose residues. The activities of the mixture of synthesized trimethylquercetin and tetramethylquercetin (TMQ) have been also analyzed. DMQ is relatively non-toxic as compared with quercetin. Both DMQ and TMQ inhibit the reproduction of flu, herpes, and hepatitis C viruses and decrease expression of p24 HIV. When rhamnose was cleaved off, DMQ activity against flu virus and bovine
diarrhea virus *in vitro* is eliminated while deglycosylated DMQ is still active against herpes virus and HIV. We have assayed DMQ and TMQ *in vitro* in models of transcription (DNA-dependent RNA polymerase of T7 phage) and replicative (Taq DNA-polymerase) complexes. The substances inhibit effectively RNA synthesis in transcription model complex with IC₅₀ for DMQ and TMQ being 4.0 µg/mL and 1.0 µg/mL, respectively. Synthesis of DNA fragments by Taq DNA-polymerase in PCR setting is also inhibited requiring higher concentrations of DMQ or TMQ. Both DMQ and TMQ at high doses of 150 µM inhibit growth of Jurkat cells inducing apoptosis in a fraction of cell population. Apoptosis induced by these substances involves caspase-9 activation. Therefore, both DMQ and TMQ possess the broad spectrum of antiviral activity with low toxicity and high selectivity index. The multiple intracellular targets of these substances have been demonstrated. The substances under study may be promising in development of novel antiviral drugs.

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**Synthesis & Antiviral activity of some Ruthenium (II) Complexes**

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Thirteen ruthenium complexes of the type Ru(L₂)(L₃)²⁺ have been prepared by reacting Ru(L₂)Cl₂ (where L=2,2'-bipyridine (bpy)/ 1,10-phenanthroline (phen)/ dimethylsulfoxide (DMSO)) with ligands L₂= BT, HBT, FCI-BT (RB), FCI-HBT, IINH, NO₂-MPC, OCH₃-MPC, DM-MPC, Cl-MPC (where BT= benzoiazole, HBT= hydrazinobenzoiazole, fluoro-chloro benzothiazole, fluoro-chloro-2-hydrazino benzothiazole, IINH= N-2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-4-carboxyhydrazide, NO₂-MPC= N(4-nitrophenyl)- methylidene-pyridine-4-carboxyhydrazide, OCH₃-MPC= N(4-methoxyphenyl)methylidene-pyridine-4-carboxyhydrazide, DM-MPC= N(4-dimethylaminophenyl)methylidene-pyridine-4-carboxyhydrazide, Cl-MPC= N(4-chlorophenyl)methylidene-pyridine-4-carboxyhydrazide. Structures of all these complexes established by IR, ¹H-NMR, & Mass spectroscopy. All compounds were tested for cytotoxicity & antiviral activity against varicella-zoster virus (VZV) in human embryonic lung (HEL) cells cytomegalovirus in human embryonic lung (HEL) cells, Herpes Simplex Virus-1 (KOS), Herpes Simplex Virus-2 (G), Vaccinia Virus, Vesicular Stomatitis Virus & Herpes Simplex Virus-1 TK-KOSACVr and compared with standard Brivudin, Cidofovir, Acyclovir and Ganciclovir. Ligand namely RB showed antiviral activity at 71.3 µM against TK-VZV strain on comparison to standard acyclovir (89 µM) and brivudin (60 µM). Among the tested complexes RDB 3 [Ru(DMSO)2(FCl-HBT)Cl2], TKA 3 Ru(bpy)2(NO₂-MPC) & TKA 6 Ru(bpy)2(Cl-MPC) showed potent antiviral activity against Vesicular stomatitis virus (41.9, 40.9 and 36.2 µM), Coxsackie virus B4 (42.1, 35.3 & >100 µM) on comparison to DS-5000 (>100 µM, 80.5), (S)-DHPA (>250 µM) and ribavirin (3.6, 13.9 µM) respectively.

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**SYNTHESIS AND ANTIVIRAL ACTIVITY OF SUBSTITUTED PYRIMIDINES**

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Human immunodeficiency virus (HIV) is a widespread virus that causes a progressive failure of the immune system. Currently there are more than 25 drugs approved by the FDA for the treatment of this infection. Their
combinations during highly active antiretroviral therapy (HAART) in many cases allow for a sustained virologic response and an increase in the life span of the patients. Non-nucleoside inhibitors of HIV reverse transcriptase (NNRTI) are widely used during HAART in combination with other types of drugs. Their use may be limited by side-effects as well as by the occurrence of drug-resistant HIV strains, which are also often observed in treatment-naïve HIV carriers. Therefore, development of novel non-toxic NNRTI active against various HIV strains presents a goal of particular importance. Here we present a series of substituted pyrimidines as NNRTI that displayed profound anti-HIV activity. The first group of compounds was comprised of \( \text{N}^1 \)-substituted pyrimidines linked to a benzophenone fragment. These inhibitors displayed antiviral activity at nanomolar concentrations, acting as RT inhibitors. Unfortunately, they were much less active against a mutant HIV-1 strain bearing K103N/Y181C substitutions. We revealed that this loss of activity was partially due to their low solubility in aqueous media. \( \text{N}^1 \)-substituted \( \text{N}^1 \)-benzyluracils represent the second group of inhibitors. They also displayed notable anti-HIV activity, although not as pronounced as the benzophenones. SAR studies revealed that \( \text{N}^1 \)-substituted \( \text{N}^1 \)-benzyluracils could be regarded as analogues of benzophenone-containing NNRTI – i.e. benzophenoneoxyethylpyrimidines (presented above) or GF128590 and related compounds, developed by GlaxoSmithKline. Future SAR studies may allow development of more effective anti-HIV agents basing on the compounds presented herein. Acknowledgments. This work was supported by State Contract 11.519.11.2192 of Russian Federation, and by grants of RFBR (10-04-00056) and of President of Russian Federation (MK-5035.2011.4).

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**Inhibition of hepatitis C virus replication and viral helicase by ethyl acetate extract of the marine feather star *Alloeocomatella polycladia***

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Hepatitis C virus (HCV) is a causative agent of acute and chronic hepatitis, leading to the development of hepatic cirrhosis and hepatocellular carcinoma. The current standard therapy with interferon and ribavirin exhibits a sustained virologic response rate of only about 50%. Thus, 5 candidates for other anti-HCV agents have been needed for the complete eradication of the virus from hepatitis C patients. HCV nonstructural protein 3 (NS3), which exhibits RNA helicase and protease activities, plays a crucial role in viral RNA replication. In this study, we prepared extracts from 61 marine organisms and screened them by an *in vitro* fluorescence assay targeting NS3 helicase to identify effective candidates for anti-HCV agents. An ethyl acetate-soluble fraction of the feather star *Alloeocomatella polycladia* exhibited the strongest inhibition of NS3 helicase activity, with an IC50 of 11.7 \( \mu \)g/ml. The extract of *A. polycladia* inhibited interaction between NS3 and RNA but not ATPase of NS3. Furthermore, the replication of the replicons derived from three HCV strains of genotype 1b in cultured cells was suppressed by the extract of *A. polycladia*. Treatment with this extract resulted in an EC50 value of 23 to 44 \( \mu \)g/ml, which is similar to the IC50 value of the NS3 helicase assay. The extract did not induce interferon or inhibit cell growth. These results suggest that the unknown compound(s) included in *A. polycladia* can inhibit HCV replication by suppressing the helicase activity of HCV NS3. This study may present a new approach toward the development of a novel therapy for chronic hepatitis C.
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Comparative analysis of cytotoxicity of fluorine-containing heterocyclic compounds in lymphoblastoid and monolayer cell cultures


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The purpose of this study was to investigate the cytotoxicity new synthesized fluorine-containing heterocyclic compounds in various cell cultures. It was used three cell cultures in this study: Raji –human B- lymphocytes, RK-13 - epithelial cells of rabbit carcinoma, MDBK - calf kidney epithelial cells. The fluorine-containing derivatives of 1,2,3-triazoles are diheterocyclic compounds that contain 1,2,3-triazole as a nucleobase with various substituents: residues of trifluoromethyl (substances \(^1\) 1, \(^1\) 2, \(^1\) 4, \(^1\) 6, \(^1\) 8), perfluoropropyl (\(^1\) 3, \(^1\) 5, \(^1\) 7, \(^1\) 9), thiosulphate (all compounds) and a glycoside fragment is represented by fragments of 3-chloro-tetrahydrofuran (\(^1\) 4, \(^1\) 5), 3-chloro-tetrahydropyran (\(^1\) 2, \(^1\) 3), dihydrofuran (\(^1\) 8 and \(^1\) 9) and dihydropyran (\(^1\) 6, \(^1\) 7). Cytotoxicity of investigated compounds was determined by using the calorimetric MTT method. It was showed that substances \(^1\) 2, \(^1\) 9 were the most toxic for all three lines of cell cultures, their CC\(_{50}\) were within 100 - 280 \(\mu\)g/ml. The high toxicity of these drugs may be associated with the presence of trifluoromethyl (\(^1\) 2) and perfluoropropyl (\(^1\) 9) groups in their triazol fragments, and 3-chloro-tetrahydrofuran and dihydrofuran in \(^1\) 2 and \(^1\) 9 accordingly. The substance \(^1\) 4 was more toxic for Raji cells, it CC\(_{50}\) was 389 \(\mu\)g/ml, and less toxic for MDBK – 887 \(\mu\)g/ml and for RK-13 - 560 \(\mu\)g/ml. The compound \(^1\) 1 showed high toxicity to RK-13 cells, it CC\(_{50}\) was 190 \(\mu\)g/ml, whereas for other cells it was less toxic - 460 \(\mu\)g/ml. The compounds \(^3\) 3 and \(^1\) 5 containing perfluoropropyl residue had high CC\(_{50}\) - 500 - 1000 \(\mu\)g/ml compared with compounds which didn’t contain this group. This dependence was observed among the substances containing 3-chloro-tetrahydrofuran and 3-chloro-tetrahydrofuran. The group of substances containing dihydrofuran and dihydropyran, which had trifluoromethyl residue in triazole fragment, showed higher CC\(_{50}\). \(^1\) 6 - 540, \(^1\) 8 - 1000 \(\mu\)g/ml compared with substances without this residue \(^7\) 7 - 225, \(^1\) 9 - 700 \(\mu\)g/ml for test cell cultures. Thus, the substances \(^1\) 3, \(^1\) 5, \(^1\) 6, \(^1\) 7 were determined as perspective for further study of their antiviral activitie.

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Efficacy Of Tranylcypromine In Murine Models Of Human Herpes Simplex Virus

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Chromatin modulation is a key regulatory component of the lytic and latency reactivation cycles of the \(\alpha\)-herpesviruses (HSV and VZV). Studies of the molecular mechanisms involved in this regulation have identified a set of histone H3-Lysine 9 demethylases that are required for the initiation of the viral gene expression program. Tranylcypromine (TCP), a monoamine oxidase inhibitor (MAOI), restricts \(\alpha\)-herpesvirus immediate early (IE) gene expression during lytic infection via inhibition of the histone H3-lysine 9 demethylase LSD1 (lysine-specific demethylase-1). LSD1 inhibitors, including MAOIs, also function to repress viral reactivation from latency in a mouse ganglia explant model. In vivo, MAOIs limit herpes simplex virus (HSV) primary infection and accumulation of viral genomes in sensory ganglia. In these studies, mice were pretreated orally twice daily with TCP (7.5 mg/kg) for 7 days and infected with HSV-2, strain MS at an LD\(_{50}-LD_{90}\). Control infected groups were treated with Vehicle or Acyclovir (ACV 50 mg/kg). HSV-2 \(\log_{10}\) genome equivalents were determined by qPCR analyses of samples of lung, liver, spleen, kidney, sensory ganglia, and brain sections taken on Day 5 and 10 from TCP and ACV treated mice and compared to samples from Vehicle treated mice. The results demonstrate that both TCP and ACV were effective at reducing viral genome copy numbers in lung, liver, spleen, kidney, cerebral cortex, olfactory bulbs, cerebellum,
pons/medulla, diencephalon or trigeminal ganglia by 0.5 to 2 logs. In a second set of studies, mice implanted with 5, 2.5 or 1.25 mg of time-release TCP pellet formulations and infected with an LD_{50} of HSV-2, also exhibited a dose dependent reduction in trigeminal ganglia HSV-2 copy numbers on Day 3 and 5 post-infection. The results clearly demonstrate the efficacy of TCP and other LSD1 inhibitors as anti-HSV therapies and warrant investigation into the impacts on viral shedding, alone and in combination with other therapies. Work supported by HHSN272201000027I, NIAID, NIH to the University of Alabama at Birmingham (UAB).
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Invitation to the Twenty-Sixth International Conference on Antiviral Research
San Francisco, California, USA
Saturday, May 11 – Wednesday, May 15, 2013

The 26th International Conference on Antiviral Research (ICAR), hosted by the International Society for Antiviral Research (ISAR), will take place in San Francisco at the Hyatt Regency San Francisco, California, USA. The conference will begin on Saturday, May 11th, 2013, and will conclude on Wednesday, May 15th, 2013.

The ICAR provides a truly interdisciplinary view of cutting-edge antiviral drug research and development that should be of interest to chemists, biologists, and clinicians. The meeting serves as a forum at which investigators involved in basic, applied, and clinical research worldwide can meet to review recent developments in all areas of antiviral research and development. Specific topics to be covered in the program include: medicinal chemistry, new target identification, biochemistry and mechanism of action, assay development, in vitro evaluation, animal models, pharmacokinetics, toxicology, and clinical trials. Within these areas of interest, there will be invited overview speakers, oral presentations, and poster presentations.

San Francisco is often called "Everybody’s Favorite City," a title earned by its scenic beauty, cultural attractions, diverse communities, and world-class cuisine. Measuring 49 square miles, this very walkable city is dotted with landmarks like the Golden Gate Bridge, cable cars, Alcatraz and the largest Chinatown in the United States. A stroll of the City’s streets can lead from Union Square to North Beach to Fisherman’s Wharf, with intriguing neighborhoods to explore at every turn. Views of the Pacific Ocean and San Francisco Bay are often laced with fog, creating a romantic mood in this most European of American cities. San Francisco is an easy flight from most of North America and Asia and usually requires minimal connections from Europe. This makes San Francisco an idea location for the 26th ICAR.

Visit the ISAR Web site at www.isar-icar.com to learn more about the 26th ICAR, such as hotel accommodations, abstract submittals, and preliminary programs beginning September 2012. If you have any questions, please do not hesitate to contact the ISAR/ICAR Office at 202-973-8690 or by email at ISAR@courtesyassoc.com.