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Programs and Abstracts

The Seventeenth International Conference on Antiviral Research

Sponsored by:

The International Society for Antiviral Research

Hilton El Conquistador Hotel

Tucson, AZ, USA

May 2–6, 2004

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Local Organizing Committee

Leroy B. Townsend

Introduction to Sponsor

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 17th year of existence, and has about 800 members representing 30 countries. For membership application forms or further information, please contact Dr. Amy Patick, Secretary, ISAR; Agouron Pharmaceuticals, Inc., Department of Virology, 4245 Sorrento Valley Blvd., San Diego, CA 92121; Ph. (858) 622-3117; Fax (858) 622-5999; E-mail amy.patick@pfizer.com. Membership application forms will also be available at the Conference Registration desk, or from our website www.isar-icar.com.

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Additional Support provided by:
Office of AIDS Research, National Institutes of Health, Bethesda, MD, USA

Satellite Symposium

Clinical Update on Antiviral Drugs

Sunday, May 2, 2004, 1 p.m. to 6 p.m.
Turquoise Ballroom
Hilton El Conquistador Hotel

Reception, 6 p.m. to 8 p.m.
Sunset Point
Hilton El Conquistador Hotel

Social Functions:

Opening Reception:

Monday, May 3, 2004, 7 p.m. to 9 p.m.
Poolside
Hilton El Conquistador Hotel

Conference Banquet:

Wednesday, May 5, 2004, 7 p.m. to 10 p.m.
Turquoise Ballroom
Hilton El Conquistador Hotel

Final Program

Seventeenth International Conference on Antiviral Research

Sponsored by:

The International Society for Antiviral Research

Hilton El Conquistador Hotel

Tucson, AZ, USA

May 2–6, 2004

Monday, May 3

Oral Session I: Retrovirus Infections

Chairpersons: Masanori Baba and Jan Balzarini

8:30 a.m. Welcome—John C. Drach, President ISAR
Earl R. Kern, Chair, ISAR Conference Committee

8:45 Plenary Speaker – Elion Award – 2003
John C. Martin, Gilead Sciences, Foster City, CA, USA
"A Brief History of Nucleotide Antivirals"

9:15 1. Nucleoside Triphosphate Mimics (P3Ms) and Their Prodrugs (P3M-PDs): A New Class of Agents with Potent Activity Against Resistant HIV-1.
N. Boyle, P.D. Cook, G. Wang, T.W. Bruice, J. Leeds, J. Brooks, P. Fagen, V. Rajwanshi, M. Prhavc, T. Hurd, and F. Chen.
Biota, Inc., Carlsbad, CA, USA.

9:30 2. Direct Measurement of Antiviral Nucleoside Monophosphate Delivery from Phosphoramidate Pronucleotides by Stable Isotope Labeling and LC-ESI-MS/MS.
C.R. Wagner, J. Kim, and T.-f. Chou.
University of Minnesota, Minneapolis, MI, USA.

9:45 3. Synergistic Anti-HIV Activity of Zinc-Finger Inhibitory Molecules Used in Combination with a Variety of Other Anti-HIV Agents.
R.W. Buckheit Jr., J. Russell, T.L. Hartman, J.K. Inman, M. Schito, A. Goel, E. Appella, and J.A. Turpin.
GeneLogic, Inc., Gaithersburg; Southern Research Institute, Frederick; National Cancer Institute, Bethesda; and The Henry M. Jackson Foundation, Rockville, MD, USA.

10:00 Break

10:30 4. Discovery of a Novel Class of Small-Molecule HIV Entry Inhibitors that Target the gp120-binding Domain of CD4.
Q. Yang, P. Roberts, W. Zhu, A. Stephen, J. Adelsberger, M. Currens, Y. Feng, R. Fisher, A. Rein, R. Shoemaker, and S. Sei.
SAIC-Frederick; and NCI-Frederick, MD, USA.

10:45 5. HIV-l Induced AIDS is an Allergy and the Allergen is the Shed gp120.
Y. Becker.
The Hebrew University of Jerusalem, Jerusalem, Israel.

11:00 6. Multiple Mutations in Human Immunodeficiency Virus-l Integrase Confer Resistance to the Phase I/II Clinical Trial Drug S-1360.
M. Witvrouw, V. Fikkert, C. Pannecouque, B. Van Remoortel, E. De Clercq, and Z. Debyser.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; and The European TRIoH Consortium.

11:15 7. Ribonucleotide Reductase Inhibitors Didox and Trimidox Compared to Hydroxyurea to Inhibit Retrovirus Replication Alone or in Combination with Abacavir, ddl or Tenofovir.
R. Sumpter, C. Mayhew, M. Inayat, T. Sugg, P. Tsai, H. Elford, and V. Gallicchio.
University of Kentucky, Lexington, KY; University of Cincinnati, Cincinnati, OH; and Molecules for Health Inc., Richmond, VA, USA.

11:30 149. Are Cholinesterases Inhibited by cycloSal Nucleotides
C. Meier, C. Ducho, and J. Balzarini.
University of Hamburg, Hamburg Germany: and Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium.

12:00 Lunch

Monday, May 3

Oral Session II: Hepadnaviruses, Retroviruses, West Nile Virus

Chairpersons: Tim Block, Chris Meier

1:30 p.m. Plenary Speaker—Prusoff Award
Fabien Zoulim, Institute Universitaire de France, Lyon, France.
"Mechanisms of Viral Persistence and Drug Resistance in Hepadnavirus Infections."

2:00 8. Generation of Novel Stable Cell Lines for Cross-Resistance Testing of Anti-HBV Compounds by Real-time PCR in a 96 Well Format.
W.E. Delaney IV, H. Yang, A. Sabogal, X. Qi, M. Miller, and S. Xiong.
Gilead Sciences, Foster City, CA, USA.

2:15 9. Long Term Incidence and the Molecular Basis of Hepatitis B Resistance to Adefovir Dipivoxil (ADV).
C.S. Gibbs, X. Qi, H. Yang, W.E. Delaney IV, K. Das, E. Arnold, V. Thibault, A. Snow, Y. Zhu, M. Curtis, S. Hadziyannis, C.E. Westland, C.L. Brosgart, S. Arterbum, M.D. Miller, and S. Xiong.
Gilead Sciences, Foster City, CA, USA; Rutgers University, Piscataway, NJ, USA; Hospital Pitie-Salpetriere, Paris, France; and Henry Dunant Hospital, Athens, Greece.

2:30 10. Novel 8-Substituted Dipyridodiazepinone Inhibitors with Broad-Spectrum Activity Against NNRTI-Resistant HIV-1.
C. Yoakim, P.R. Bonneau, R. Déziel, L. Doyon, J. Duan, I. Guse, B. Haché, S. Landry, E. Malenfant, J. Naud, W.W. Ogilvie, J.A. O'Meara, R. Plante, B. Simoneau, B. Thavonekham, M. Bös, and M.G. Cordingley.
Boehringer Ingelheim (Canada), Ltd., Laval, Que., Canada.

2:45 11. Specific Killing of HIV Infected Cells by Cyclin Dependent Kinase Inhibitors.
J. Hesselgesser, C.S. Gibbs, and R. Shibata.
Gilead Sciences, Foster City, CA, USA.

3:00 12. HIV-1 Resistance to Binding/Fusion Inhibitors.
J.A. Esté and B. Clotet.
Hospital Universitari, Badalona, Spain.

3:15 13. Delivery of Anti-Viral Immunoglobulins into the CNS for Treatment of Encephalitic Viruses.
A.L. Olsen and J.D. Morrey.
Utah State University, Logan, UT, USA.

3:30 14. Evidence for Transplacental Infection of Rodent Fetuses with West Nile Virus.
J.G. Julander, J.D. Morrey, A.L. Olsen, C.W. Day, and R.W. Sidwell.
Utah State University, Logan, UT, USA.

3:45 15. Rapid Development of an Antisense Phosphorodiamidate Morpholino Oligomer for the Treatment of West Nile Virus.
P.L. Iversen, D. Stein, A. Kroeker, V. Arora, E. Barklis, A. Hill, A. Smith, and R. Wallace.
AVI BioPharma, Corvallis; Oregon Health Sciences University, Portland; Oregon State University, Corvallis, OR; and Milwaukee County Zoo, Milwaukee, WI, USA.

Monday, May 3

Poster Session 1: Retrovirus, Hepatitis Virus Infections

4 p.m.–6 p.m.

16. Synthesis of Novel CADA Compounds and Their Anti-HIV and CD4 Down-Modulation Activities.
S. Anugu, N. Duffy, K. Dey, Q. Jin, M.F. Samala, A. Sodoma, T.W. Bell, K. Vermeire, E. De Clercq, and D. Schols.
University of Nevada, Reno, NV, USA; and Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium.

17. Aminoglycoside-arginine Conjugates: Chemical Barriers of HIV-1 Entry.
A. Lapidot and G. Borkow.
Weizmann Institute of Science, Rehovot, Israel.

18. Synthetic Polymeric Antagonists for Receptors of HIV-1 Entry into Human Cells. A.V. Serbin, I.V. Timofeyev, O.L. Alikhanova, N.G. Perminova, V.N. Semerikov, N.N. Karpyshev, I.V. Ermakov, D.I. Timofeyev, T.S. Grebinic, A.Y. Bakulina, L. Margolis, and Y.Y. Kiseleva.
Health RDF; Topchiev Inst., Moscow; SRC VB "Vector", Kolt'sovo, Russia; and NICHHD, NIH, Bethesda, MD, USA.

19. RD51643, A Novel Diketo Acid Which Selectively Inhibits the HIV-l Multiplication in Cell-Based Assays and the Ribonuclease H Activity in Enzyme Assays.
E. Tramontano, F. Esposito, R. Badas, R. Costi, R. Di Santo, and P. La Colla.
University of Cagliari, Cagliari, Italy; and University of Rome "La Sapienza", Rome, Italy.

20. 4′-Ethynyl-d4T, a Novel Nucleoside Inhibitor of HIV-l with Unique Properties.
T. Nitanda, K. Haraguchi, H. Tanaka, Y.-C. Cheng, and M. Baba.
Kagoshima University, Kagoshima; Showa University, Tokyo, Japan; and Yale University, New Haven, CT, USA.

21. Susceptibility of HIV-2, SIV and SHIV to Various Anti-HIV-1 Compounds: Implications for Treatment and Postexposure Prophylaxis.
M. Witvrouw, C. Pannecouque, W.M. Switzer, T.M. Folks, E. De Clercq, and W. Heneine.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; and Centers for Disease Control and Prevention, Atlanta, GA, USA.

22. Inhibition of HIV-1 Replication Using a Single Transcriptional Unit that Expresses Decoy and siRNAs in Mammalian Cells.
H. Takaku, J.S. Barnor, N. Miyano-Kurosaki, K. Ishikawa, N. Yamamoto, and M. Osei-Kwasi.
Chiba Institute of Technology, Narashino, Chiba; National Institute of Infectious Diseases, Japan; Noguchi Memorial Institute for Medical Research, Ghana; and Tokyo Medical and Dental University, Tokyo, Japan.

23. Inhibition of Human Immunodeficiency Virus Type 1 via HIV-l Dependent Anti-gene Expression System.
Y. Habu, T. Nagawa, N. Miyano-Kurosaki, and H. Takaku.
Chiba Institute of Technology, Narashino, Chiba, Japan.

24. Optimization of Unique Thioesters as Antiviral Agents that Target HIV-1 Nucleocapsid Protein (NCp7).
T.L. Hartinan, R.W. Buckheit Jr., J.A. Turpin, J.K. Inman, P. Srivastava, T. Hara, R. Fattah, M. Schito, and E. Appella.
GeneLogic, Inc., Gaithersburg; The Henry M. Jackson Foundation, Rockville; and National Cancer Institute, Bethesda, MD, USA.

25. Anti-HIV Activity Profile of a Novel CCR5 Inhibitor, AMD887, in Combination with the CXCR4 Inhibitor AMD070.
D. Schols, K. Vermeire, S. Hatse, K. Princen, E. De Clercq, G. Calandra, S. Fricker, K. Nelson, J. Labrecque, D. Bogucki, Y. Zhou, R. Skerlj, and G. Bridger.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; and AnorMED, Inc., Langley, BC, Canada.

26. Cyclic Dinucleotides as Potential Inhibitors of the 3′-Processing Step of HIV-1 DNA Integration.
V. Nair, G. Chi, and B.I. Seo.
University of Georgia, Athens, GA, USA.

27. Activity Assays of HIV-1 Integrase Using Microtiter Plate Technology.
S. John and C.B. Jonsson.
University of Alabama at Birmingham; and Southern Research Institute, Birmingham, AL, USA.

28. Inhibition of Late Stage Events in HIV Replication: Therapeutic Compounds with Effects on Viral RNA Synthesis.
T.B. Parsley, T.L. Hartman, T.L. Brenner, J.A. Turpin, and R.W. Buckheit Jr.
GeneLogic, Inc., Gaithersburg, MD; University of Arizona College of Pharmacy, Tucson, AZ; and The Henry M. Jackson Foundation, Rockville, MD, USA.

29. Intracellular Neutralization of HIV-l Replication by Immunoglobulin A.
A. Wright, X. Gao, L. Kulick, H. Yan, M. Lamm, and Y. Huang.
Case Western Reserve University, Cleveland, OH, USA.

30. Changes in HIV Antiviral Phenotypic Drug Resistance During the HAART Era.
G. Hess, C. Nelson, and J. Seiders.
Surveillance Data, Inc., Plymouth Meeting, PA, USA.

31. Selection and Characterization of Virus Strains Resistant to a Novel Pyrimidinedione Nonnucleoside Inhibitor of HIV-1 and HIV-2.
K.M. Watson, T.B. Parsley, and R.W. Buckheit Jr.
GeneLogic, Inc., Gaithersburg, MD, USA.

32. Discovery and Characterization of Nonnucleoside Reverse Transcriptase Inhibitors Related to SJ-3366 with Efficacy Against Viruses Possessing the K103N Mutation in the Viral Reverse Transcriptase.
K.M. Watson, T.L. Hartman, S.-G. Chung, E.-H. Cho, and R.W. Buckheit Jr.
GeneLogic, Inc., Gaithersburg, MD, USA; and Samjin Pharmaceutical Co., Ltd., Seoul, Korea.

33. Sensitive Methodology to Assess Resistance, Fitness, Replication Capacity and Compensatory Mutations of Reverse Transcriptase Inhibitor Resistant Viruses.
T.L. Hartman, K.M. Watson, T.B. Parsley, and R.W. Buckheit Jr.
GeneLogic, Inc., Gaithersburg, MD, USA.

34. Novel Tyrosine-Derived HIV Protease Inhibitors Possessing Potent Anti-HIV Activity and Remarkable Inverse Antiviral Resistance Profiles.
J.F. Miller, M. Brieger, E.S. Furfine, R.J. Hazen, and A. Spaltenstein.
GlaxoSmithKline, Research Triangle Park, NC, USA.

35. HIV-1-Resistance to ADS-J1.
M. Armand-Ugón, I. Clotet, S. Jiang, B. Clotet, and J.A. Esté.
Hospital Universitari, Barcelona, Badalona, Spain; and The New York Blood Center, New York, NY, USA.

36. Variability in the Use of HIV Phenotypic Resistance Testing by Specialty of Practitioner.
G. Hess, C. Nelson, and J. Seiders.
Surveillance Data, Inc., Plymouth Meeting, PA, USA.

37. Mechanism of Anti-HIV Activity of Dioxolane Nucleosides Against 3TC-Resistant HIV-1 Reverse Transcriptase-Molecular Modeling Approach.
C.K. Chu, R.F. Schinazi, and Y.H. Chong.
The University of Georgia, Athens; and Emory University/VA Medical Center, Decatur, GA, USA.

38. Virus Resistance to the CXCR4 Inhibitor AMD070 Develops Slowly and Does Not Induce a Co-Receptor Switch.
K. Vermeire, S. Hatse, K. Princen, E. De Clercq, G. Calandra, R. Skerlj, G. Bridger, and D. Schols.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; and AnorMED, Inc., Langley, BC, Canada.

39. Anabolism of Amdoxovir: Phosphorylation of Dioxolane Guanosine and its 5′-Phosphates by Mammalian Phosphotransferases.
J. Feng, W Parker, M. Krajewski, D. Deville-Bonne, M. Veron, P. Krishnan, Y. Cheng, and K. Borroto-Esoda.
Gilead Sciences, Durham, NC; Southern Research Institute, Birmingham, AL, USA; Institut Pasteur, Paris, France; and Yale University, New Haven, CT, USA.

40. Evaluation of Virologic Response and Resistance Profile in Treatment Experienced Patients Receiving Amdoxovir.
J. Waters, J. Harris, G. Shen, F. Rousseau, K. Borroto-Esoda, and N. Adda.
Gilead Sciences, Inc., Durham, NC, USA.

41. MW-150, A Potent HIV-1 Inhibitor Suitable for use in Microbicides.
B. Öberg, D. Böttiger, D. Phillips, J.F. Romeo, M. Thom, L. Vrang, and H. Zhang.
Medivir AB, Huddinge, Sweden; and Population Council, New York, NY, USA.

42. Design and Preclinical Development of Dendrimer Based Topical Microbicides for HIV and STI Prevention.
T. McCarthy, P. Karellas, S. Henderson, M. Giannis, D. O'Keefe, B. Matthews, B. Braggs, J. Paull, G. Heery, G. Krippner, and G. Holan.
Starpharma Limited, Melbourne, Vic., Australia.

43. In Vitro Strategies for the Inhibition of Sexually Transmitted Diseases: Preclinical Development of Topical Mono- and Combination Microbicide Therapies.
K.M. Watson, T.B. Parsley, and R.W. Buckheit Jr.
GeneLogic, Inc., Gaithersburg, MD, USA.

44. Topical Microbicide Therapies Involving the use of Nonnucleoside Reverse Transcriptase Inhibitors: Combination Therapy and Resistance.
K.M. Watson, T.L. Hartman, and R.W. Buckheit Jr.
GeneLogic, Inc., Gaithersburg, MD, USA.

45. Novel Acyclic Nucleoside Phosphonate Analogues as Potent and Selective Anti-HBV Agents.
C. Ying, A. Holý, D. Hockova, E. De Clercq, and J. Neyts.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; and Academy of Sciences of the Czech Republic, Praha, Czech Republic.

46. Investigation into the Ability of GBV-B to Replicate in Various Immortalized Cell Lines.
V.E. Buckwold, B. Collins, and J. Wei.
Southern Research Institute, Frederick, MD, USA.

47. A Comparative Analysis of Two Hepatitis B Virus (HBV) Viral Load Assays: Real-time PCR versus Hybrid Capture.
B. Chappell, M. Curtis, A. Snow, Y. Zhu, C. Wakeford, and J. Harris.
Gilead Sciences, Inc., Durham, NC, USA.

48. Highly Sensitive and Accurate Real-time PCR Assay for Hepatitis B Viral Load Quantification.
M. Curtis, Y. Zhu, and M. Jackson.
Gilead Sciences, Inc., Durham, NC, USA.

49. Molecular Modelling of Hepatitis B Virus Polymerase: Comparison of Adefovir, Entecavir and Lamivudine Resistance.
A. Bartholomeusz, S. Locarnini, D. Colledge, A. Ayres, M. Kuiper, and D. Chalmers.
Victorian Infectious Diseases Reference Laboratory, Melbourne; Victorian Partnership for Advanced Computing, Melbourne; and Vic College Pharmacy, Parkville, Vic., Australia.

50. Ribavirin and Mycophenolic Acid Potentiate the Activity of Entecavir against Hepatitis B Virus.
C. Ying, E. De Clercq, R. Colonno, and J. Neyts.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; and Bristol-Myers Squibb, Wallingford, CT, USA.

51. Long-term Adefovir Dipivoxil (ADV) Demonstrates Sustained Efficacy in Chronic Hepatitis B (CHB).
C.L. Brosgart, S. Hadziyannis, Y. Benhamou, S. Arterburn, J. Ma, B. Lu, S. Xiong, M. Wollman, C. James, C.G. Chang, and G. Currie.
Gilead Sciences, Inc., Foster City, CA, USA; Henry Dunant Hospital, Athens, Greece; and Hopital Pitie-Salpetriere, Paris, France.

52. Characterisation and Functional Analysis of Hepatitis B Virus Mutants Selected in Patients with Severe Hepatitis During Long Term Lamivudine Therapy.
A. Bartholomeusz, N. Warner, A. Ayres, G. Thompson, R. Edwards, V. Sozzi, N.W.Y. Leung, M. Kuiper, D. Chalmers, and S. Locarnini.
Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia; Prince of Wales Hospital, Hong Kong SAR, China; Victorian Partnership for Advanced Computing; and Vic College Pharmacy, Parkville, Vict., Australia.

53. The Virological Basis of the Hypersensitivity of HBV, HCV and Pestiviruses Sensitivity to Glucosidase Inhibitors.
T.M. Block, E. Sinisek, A.S. Mehta, P. Norton, B. Gu, J. Gong, and T. Zhou. Jefferson Medical College, Doylestown, PA, USA.

54. Glycolipid Mimetics Induce an Innate Host Response and have Anti-HBV and HCV Activity, In Vitro.
T.M. Block, B. Gu, X. Lu, B. Conyers, L. Wang, B. Tennant, B. Korba, R. Morriarty, A.S. Mehta, and R.A. Dwek.
Jefferson Medical College, Doylestown, PA, USA.

55. A Cell-based Assay for Screening of Potential Antiviral Agents Against HCV.
A. Zauberman, E. Ilan, S. Aviel, D. Slama, I. Tsionas, K. Shochat, D. Landstein, R. Eren, T. Miyamura, S. Nagamori, and S. Dagan.
XTL Biopharmaceuticals, Ltd., Rehovot, Israel; and National Institute of Infectious Diseases, Tokyo, Japan.

56. Development of a Sensitive and Rapid Real-Time RT-PCR Assay using Lightcycler for Quantitative Analysis of Hepatitis C Virus RNA.
Y. Gong, T. Tam, I. Gadawski, and S. Sacks.
Viridae Clinical Sciences, Inc.; The University of British Columbia; and Health Sciences Mall, Vancouver, BC, Canada.

57. Chimeric HCV Replicons and Distinct Cellular Requirements for HCV and BVDV Replicon Replication.
J.A. Lemm, M. Liu, H. Yigit, R.E. Rose, R. Fridell, D.R. O'Boyle II, R. Colormo, and M. Gao.
Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA.

58. High-Throughput Specificity HCV Replicon Screening Assay.
J.A. Lemm, D.R. O'Boyle II, L. Valera, P.T. Nower, J.-H. Sun, K. Rigat, and M. Gao.
Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA.

59. Screening for Hepatitis C Virus Antiviral Activity with a Cell-Based Secreted Alkaline Phosphatase Reporter Replicon System.
N. Bourne, R. Pyles, M.-K. Yi, R. Veselenak, M. Davis, and S. Lemon.
The University of Texas Medical Branch, Galveston, TX, USA.

60. Replication-competent Hepatitis C Virus Subgenomic Replicons Containing Engineered Affinity Tags Within the NS5A Protein.
M. Gao, J.A. Lemm, M. Liu, Y. Wang, D. Qiu, R. Fridell, and D.R. O'Boyle II.
Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA.

61. Optimisation of Peptide Based Inhibitors of Full-Length Hepatitis C Virus NS3 Protease.
A. Poliakov, A. Johansson, E. Åkerblom, G. Lindeberg, A. Hallberg, and U.H. Danielson.
Uppsala University, Uppsala, Sweden.

62. Non-Nucleoside Allosteric Inhibitors of HCV NS5B Polymerase: Novel Benzimidazole Diamide Derivatives with Replicon Activity.
P.L. Beaulieu.
Boehringer Ingelheim (Canada), Ltd., Laval, Que., Canada.

63. Synergistic Inhibition of Replication of BVDV by PG 301029 Used in Combination with Ribavirin or Ribavirin and Interferon.
R.W. Buckheit Jr. And T.L. Brenner.
Gene Logic, Inc., Gaithersburg, MD; and University of Arizona College of Pharmacy, Tucson, AZ, USA.

64. In Vitro Combination Studies of VX-950 (a UCV Protease Inhibitor) or VX-497 (an IMPDH Inhibitor), Two Novel Anti-HCV Clinical Candidates, with IFN-α.
K. Lin, C. Lin, and A.D. Kwong.
Vertex Pharmaceuticals, Inc., Cambridge, MA, USA.

65. Clinical Evaluation of a Human Monoclonal Antibody Against the Envelope Protein (E2) of HCV for Prevention of HCV Infection.
E. Ilan, A. Zauberman, N. Graham, O. Nussbaum, D. Terkieltaub, E. Galun, N. Terrault, R. Eren, and S. Dagan.
XTL Biopharmaceuticals, Ltd., Rehovot; Hadassah University Hospital; and University of California at San Francisco, CA, USA.

66. Effect of Interferon-alpha and Interferon-inducers on West Nile Virus in Cell Culture and in Mouse and Hamster Animal Models.
C.W. Day, J.D. Morrey, J.G. Julander, L.M. Blatt, D.F. Smee, and R.W. Sidwell.
Utah State University, Logan, UT, USA; and InterMune, Inc., Brisbane, CA, USA.

67. Prevention of Viral Invasion by Immunocamouflage of Target Cells.
L.L. McCoy and M.D. Scott.
University of British Columbia, Vancouver, BC, Canada.

68. Effects of an HIV-l Attachment Inhibitor on the Conformation of the Viral Envelope Gp120.
H.-T. Ho, L. Fan, J. Dicker, B. McAuliffe, N.-N. Zhou, R. Fridell, J. Robinson, R. Colonno, and P.-F. Lin.
Bristol-Myers Squibb, Wallingford, CT; and Tulane University Medical School, New Orleans, LA, USA.

Tuesday, May 4

Oral Session III: Mini-symposium—What is new in emerging infections?

Chairpersons: Earl R. Kern and Richard J. Whitley

8:30 a.m.	Plenary Speaker—Elion Award—2004 Richard J. Whitley, University of Alabama at Birmingham, Birmingham, AL, USA "Acyclovir: A Springboard for Antiviral Drug Development."
9:00	Lyle Petersen, Centers for Disease Control, Ft. Collins, Co, USA. "Epidemiological and Clinical Features of West Nile Virus in North America"
9:30	Gary Clark, CDC/DHHS/NCID/VBI, San Juan, Puerto Rico, "Dengue in the Americas"
10:00	Break
10:30	Joanne Cono, Centers for Disease Control and Prevention, Atlanta, GA, USA. "Monkeypox in the U.S."
11:00	Fred Hayden, University of Virginia, Charlottesville, VA, USA. "Global Respiratory Virus Infections"
11:30	Discussion
12:00	Adjourn
12:30	Depart for Sonoran Desert Museum Tour
	Free Afternoon

Wednesday, May 5

Oral Session IV: Herpesvirus, Poxvirus Infections

Chairpersons: Graciela Andrei and Don Smee

8:30 a.m.	Plenary Speaker John Rossi, Beckman Research Institute, Duarte, CA, USA. "RNAi, Mechanisms and Anti-HIV-1 Applications."
9:00	69. Differing SARs and MOAs Emerge for the Highly Potent Anti-VZV, BCNAs, and Their Anti-HCMV dd Analogues. C. McGuigan, O. Bidet, A. Carangio, G. Luoni, G. Andrei, R. Snoeck, E. De Clercq, and J. Balzarini. Cardiff University, Cardiff, UK; and Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium.
9:15	70. Synthesis and Antiviral Activity of Certain Chlorinated Indole Nucleosides as Potent and Selective Inhibitors of HCMV Replication. J.D. Williams, J.J. Chen, J.M. Breitenbach, K.Z. Borysko, J.C. Drach, and L.B. Townsend. University of Michigan, Ann Arbor, MI, USA.
9:30	71. Comparative Antiviral Activity of Alkyl and Alkoxyalkyl Esters of Cidofovir Against HCMV Infected Cells, In Vitro. W.B. Wan, C. Hartline, E.R. Kern, and K.Y. Hostetler. Chimerix, Inc., La Jolla, CA, USA; The University of Alabama, Birmingham, AL, USA; the San Diego VA Healthcare System; and the University of California, San Diego, La Jolla, CA, USA.

9:45 72. Identification of the Mode of Action of Methylenecyclopropane Analogs using a Chemotypic Clustering Analysis.
M.N. Prichard, A.D. Williams, C.B. Hartline, Z. Yan, J. Zemlicka, W.J. Britt, and E.R. Kern.
The University of Alabama School of Medicine, Birmingham, AL, USA; and Wayne State University, Detroit, MI, USA.

10:00 Break

10:30 Invitation to 18th ICAR
ISAR Business Meeting

11:00 73. Effect of Oral Treatment with a Methylenecyclopropane Analog, ZSM-I-62, on Replication of Human Cytomegalovirus (MCMV) or Murine Cytomegalovirus (MCMV) Infection in Animal Models.
D.C. Quenelle, D.J. Bidanset, D.J. Collins, B.P. Herrod, C.B. Hartline, Z. Yan, J. Zemlicka, and E.R. Kern.
The University of Alabama School of Medicine, Birmingham, AL; and Wayne State University, Detroit, MI, USA.

11:15 74. Inhibition of Orthopoxvirus DNA Polymerases by Cidofovir Diphosphate: In Vitro Enzymatic Studies using Highly Purified Vaccinia Virus DNA Polymerase.
D.H. Evans, W. Magee, and K.Y. Hostetler.
University of Alberta, Edmonton, AB., Canada; and VA San Diego Healthcare System and University of California, San Diego, La Jolla, CA, USA.

11:30 75. A Cidofovir-Resistant Form of the Highly Virulent WR Strain of Vaccinia Virus is Cross-Resistant to Related Antiviral Agents and is Highly Attenuated for Virulence in Mice.
D.F. Smee, K.W. Bailey, A. Holý, and R.W. Sidwell
Utah State University, Logan, UT, USA; and Academy of Sciences of the Czech Republic, Prague, Czech Republic.

11:45 76. Successful Cidofovir Treatment of Smallpox-Like Disease in Variola and Monkeypox Primate Models.
J.W. Huggins, M.J. Martinez, C.J. Hartmann, L.E. Hensley, D.L. Jackson, D.F. Kefauver, D.A. Kulesh, T. Larsen, D.M. Miller, E.M. Mucker, J.D. Shamblin, M.K. Tate, C.A. Whitehouse, S.H. Zwiers, and P.B. Jahrling.
USAMRIID, Fort Detrick, MD, USA.

12:00 Lunch

Wednesday, May 5

Oral Session V: Respiratory Infections

Chairpersons: Dale Barnard and Amy Patick

1:30 p.m. Plenary Speaker
Kathryn Holmes, University of Colorado HSC, Denver, CO, USA.
“SARS Coronavirus: Targets for Antiviral Therapy.”

2:00 77. Inhibition of SARS Coronavirus In Vitro by Human Interferons.
D.L. Barnard, V.D. Hubbard, J. Burton, D.F. Smee, J.D. Morrey, and R.W. Sidwell.
Utah State University, Logan, UT, USA.

2:15 78. Inhibitory Activity of Vancomycin, Eremomycin and Teicoplanin Aglycon Derivatives Against Feline and Human (i.e. SARS) Coronaviruses.
J. Balzarini, E. Keyaerts, L. Vijgen, E. De Clercq, S.S. Printsevskaya, M. Preobrazhenskaya, and M. Van Ranst.
Rega Institute for Medical Research, K.U. Leuven; U.Z. Leuven, Leuven, Belgium; and Institute of New Antibiotics, Moscow, Russia.

2:30 79. In Vitro-Investigation of Potential Therapeutics for the Severe Acute Respiratory Syndrome (SARS).
H.W. Doerr, M. Michaelis, W. Preiser, and J. Cinatl Jr.
Johann Wolfgang Goethe University, Frankfurt, Germany.

2:45 80. Fusogenic Mechanism of SARS-Associated Coronavirus.
S. Liu, Y. He, A.K. Debnath, and S. Jiang.
New York Blood Center, New York, NY, USA.

3:00 81. Towards a Model for Small Molecule Inhibition of Virus Fusion: Photoaffinity Labeling Identifies the Binding Pocket of an RSV Inhibitor.
J. Roach, C. Cianci, D. Disehino, D. Langley, Y. Sun, K.-L. Yu, N. Meanwell, and M. Krystal.
Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA.

3:15 82. A Novel Respiratory Syncytial Virus Inhibitor.
D. Alber, L. Wilson, B. Baxter, E. Henderson, V. Dowdell, R. Kelsey, S. Keegan, R. Harris, D. McNamara, S. Bithell, N. Weerasekera, R. Harland, J. Stables, S. Cockerill, K. Powell, and M. Carter.
Arrow Therapeutics, Ltd., London, UK.

3:30 83. Effect of Conjugated Linoleic Acid on Experimental Rhinovirus Illness.
F.G. Hayden and M. O'Shea.
University of Virginia, Charlottesville, VA; and Loders Croklaan, Channahon, IL, USA.

3:45 84. Efficacy of PMEG [9-(2-phosphonylmethoxyethyl)guanine] and its Prodrug cPr-PMEDAP [9-(2-phosphonylmethoxyethyl)-*N*6-cyclopropyl-2,6-diaminopurine in Organotypic Cultures of Normal and Papillomavirus (HPV)-Positive Keratinocytes.
G. Andrei, J. Van Den Oord, G. Wolfgang, W.A. Lee, E. De Clercq, R. Snoeck.
Rega Institute for Medical Research, K.U. Leuven, Laboratory of Morphology and Molecular Pathology, K.U. Leuven, Leuven, Belgium; and Gilead Sciences, Foster City, CA, USA.

Wednesday, May 5

Poster Session II: Herpesvirus, Poxvirus, Respiratory Viruses and Other Infections

4 p.m.–6 p.m.

85. A Novel Compound IC977467 with Potent Anti-Herpesvirus Activities.
Y. Gong, Y. Lai, I. Gadawski, T. Tam. J. Hayflick, and S. Florio.
Viridae Clinical Sciences, Inc.; The University of British Columbia, Vancouver, BC, Canada; ICOS Corporation, Bothell, Washington, USA.

86. A Flow Cytometric Study on Antiviral Effect of Seaweed Polysaccharides against Herpes Simplex Viruses.
H. Wang, L.C.M. Chiu, V.E.C. Ooi, and P.O. Ang.
The Chinese University of Hong Kong, Hong Kong, China.

87. Oligonucleotides Containing the Cognate Recognition Sites for the HSV Transcriptional Regulators ICP4 and α-TIF Reduce the Expression of Target Viral Proteins and Progeny Virus Production.
K.L. Carter, J.L. Joyal, A. Chakravorty, and D.I. Israel.
PRAECIS Pharmaceuticals, Inc., Waltham, MA, USA.

88. Inhibitors of Herpes Virus and Inflammation.
G.I. Danilenko, S.L. Rybalko, T.A. Buchtiarova, V.P. Danilenko, Z.P. Omelianenko, S.V. Guzhova, and T.I. Grigrieva.
NAS Ukraine; and AMS Ukraine, Kiev, Ukraine.

89. Inhibitors of Herpes Simplex Virus Studied in a Murine Infection Model of the Immunocompromised Host.
G. Lakra and H.J. Field.
University of Cambridge, Cambridge, UK.

90. Inverse Fourier Transform as a Part of Fractal Microscope in Virus–Cell Interaction Imaging.
A.O. Fedchuk, A.S. Fedchuk, and O.P. Fedchuk.
I.I. Mechnikov Odesa National University, Odessa, Ukraine.

91. Fractal Design of Virus–Cell Dynamic System.
O.P. Fedehuk, A.S. Fedchuk, and A.O. Fedchuk.
I.I. Mechnikov Odesa National University, Odessa, Ukraine.

92. *N*-[(2-Hydroxyethoxy)methyl] Derivatives of Bicyclic Furano and Pyrrolo Pyrimidines and Their Antiviral Evaluation.
Z. Janeba and M.J. Robins.
Brigham Young University, Provo, UT, USA.

93. Bicyclic Anti-VZV Nucleosides: Thieno Analogues Bearing an Alkylphenyl Side Chain Result in a Reduction in Antiviral Activity.
A. Angell, L. Sevillano, C. McGuigan, R. Snoeck, G. Andrei, J. Balzarini, and E. De Clercq.
Cardiff University, Cardiff Wales, U.K.; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium.

94. Although the Bicyclic Nucleoside Analogues (BCNAs) are Highly Active Against Varicella-Zoster Virus (VZV), they are Inactive Against Simian Varicella Virus (SVV) Despite Efficient Phosphorylation of the BCNAs by SVV-encoded Thymidine Kinase.
R. Sienaert, G. Andrei, R. Snoeck, E. De Clercq, G. Luoni, C. McGuigan, and J. Balzarini.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Cardiff University, Cardiff, UK.

95. Characterization of the DNA Polymerase Genes of Varicella-Zoster Virus (VZV) Resistant to Foscarnet (PFA) and the 2-Phosphonomethoxyethyl (PME) Derivatives of Adenine (Adefovir, PMEA) and 2,6-Diaminopurine (PMEDAP).
G. Andrei, P. Fiten, G. Opdenakker, E. De Clercq, and R. Snoeck
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium.

96. Enhanced Metabolic Stability of Chlorinated Indole Nucleosides.
P.J. Lorenzi, J.D. Williams, J.C. Drach, L.B. Townsend, and G.L. Amidon.
University of Michigan, Ann Arbor, MI, USA.

97. Synthesis and Antiviral Activity of 9-[(3-hexadecyloxypropyl-phosphono)-1-propyloxymethyl]guanine.
J.C. Ruiz, K.A. Aldern, J. Trahan, J.R. Beadle, and K.Y. Hostetler.
VA San Diego Healthcare System and the University of California, La Jolla, CA, USA.

98. Direct Synthesis of Acyclic Nucleoside Phosphonate Alkoxyalkyl Monoesters.
J.R. Beadle, N. Valiaeva, W. Brad Wan, and K.Y. Hostetler.
San Diego Healthcare System and the University of California, La Jolla, CA, USA.

99. Synthesis and Antiviral Activity of 5-Substituted Hexadecyloxypropyl-1-[2-(phosphonomethoxy)-ethyl]cytosine (PMEC) Derivatives.
N. Valiaeva, K.A. Aldern, J. Trahan, J.R. Beadle, and K.Y. Hostetler.
VA San Diego Healthcare System and the University of California, La Jolla, CA, USA.

100. New Class of Fluoro Substituted Methylenecyclopropane Analogues of Nucleosides as Antiviral Agents: Synthesis and Antiviral Activity.
J. Zemlicka, S. Zhou, E.R. Kern, Y.-C. Cheng, J.C. Drach, and H. Mitsuya.
Wayne State University School of Medicine, Detroit, MI; University of Alabama, School of Medicine, Birmingham, AL; Yale University School of Medicine, New Haven, CT, USA; University of Michigan, Ann Arbor, MI; National Cancer Institute, Bethesda, MD, USA.

101. In Vitro Activity of Second Generation Methylenecyclopropane Analogs of Nucleosides Against Herpesvirus Replication.
N.L. Kushner, S.L. Williams-Aziz, C.B. Hartline, E.A. Harden, S. Zhou, J. Zemlicka, and E.R. Kern.
The University of Alabama School of Medicine, Birmingham, AL; Wayne State University School of Medicine, Detroit, MI, USA.

102. Use of Clustering Algorithms for Analysis of Chemotypic Data to Help Elucidate the Mechanism of Action of New Antiviral Agents.
M.N. Prichard, A.D. Williams, C.B. Hartline, W.J. Britt, and E.R. Kern.
The University of Alabama School of Medicine, Birmingham, AL, USA.

103. Cellular Uptake of Cidofovir and Various Alkoxyalkyl Esters of Cidofovir in MRC-5 Cells: Comparison with Antiviral Activity.
K.A. Aldern and K.Y. Hostetler.
VA San Diego Healthcare System and the University of California, San Diego, La Jolla, CA, USA.

104. Mucosal Immunization with a Replication-Deficient Adenovirus Vector Expressing Glycoprotein H of Murine Cytomegalovirus Induces Mucosal and Systemic Immunity.
J.D. Shanley and C.A. Wu.
University of Connecticut Health Center, Farmington, CT, USA.

105. Search and Assessment of Novel Substances Active Against Epstein–Barr Virus.
N. Nesterova, N. Dyachenko, V. Atamaniuk, A. Novik, A. Shalamai, S. Rybalko, S. Zagorodnya, and G. Baranova.
NAS of Ukraine; Ecopharm Research and Production Company; Borschanhovskyi Chemical and Pharmaceutical Plant; AMS of Ukraine, Kyiv, Ukraine.

106. Activity of Novel Acyclic Pyrimidine Nucleoside Phosphonates against Poxviruses.
G. Andrei, A. Holý, F. Dal Pozzo, A. Scagliarini, E. De Clercq, and R. Snoeck.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Academy of Sciences of the Czech Republic, Prague, Czech Republic; Alma Mater Studiorum, Bologna, Italy.

107. In Vitro Activity of Alkyl Esters of Cidofovir and Cyclic Cidofovir Against Orthopoxvirus Replication: Comparison of Activity with Alkoxyalkyl Esters.
K.A. Keith, W.B. Wan, J.R. Beadle, K.Y. Hostetler, and E.R. Kern.
University of Alabama School of Medicine, Birmingham, AL; VA San Diego Healthcare System and the University of California, San Diego, La Jolla, CA, USA.

108. HDP-(S)HPMPA: Oral Pharmacokinetics and Antiviral Activity Against Orthopoxvirus and Murine CMV Infections in Mice.
J. Trahan, D.C. Quenelle, S.L. Ciesla, E.R. Kern, and K.Y. Hostetler.
San Diego VA Healthcare System and the University of California, San Diego, La Jolla, CA, USA; The University of Alabama at Birmingham, Birmingham, AL, USA.

109. Effect of (S)-HPMPDAP and (S)-HPMPO-DAPym on Vaccinia Virus Infections in Mice.
J. Neyts, P. Leyssen, E. De Clercq, and A. Holý.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Academy of Sciences of the Czech Republic, Czech Republic.

110. A Non-Nucleoside Related to Isoniazid Exhibits Potent Anti-Orthopoxvirus Activity in Cell Culture but not in Mice.
G.I. Danilenko, S.V. Guzhova, D.F. Smee, K.W. Bailey, and R.W. Sidwell.
National Academy of Sciences of the Ukraine, Kiev, Ukraine; Utah State University, Logan, UT, USA.

111. Isolation of Vaccinia Virus (VV) Mutants Resistant to Different Acyclic Nucleoside Phosphonate Analogues (ANPs).
G. Andrei, A. Holý, P. Fiten, G. Opdenakker, E. De Clercq, and R. Snoeck.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Academy of Sciences of the Czech Republic, Prague, Czech Republic.

112. Antiviral Ribonucleosides: Inhibitors of Inosine Monophosphate Dehydrogenase.
V. Nair, S. Story, M. Gupta, and E. Bonsu.
University of Georgia, Athens, GA, USA.

113. Combinatorial Antibodies Against Orthopoxviruses.
E. Bovshik, T. Yun, E. Zhirakovskaya, V. Morozova, A. Guskov, T. Batanova, A. Ilyichev, and N. Tikunova.
State Research Centre of Virology and Biotechnology Vector, Koltsovo, Novasibirsk Region, Russia.

114. vvG1L: An Antiviral Drug Target.
D.E. Hruby, C.M. Byrd, and T.C. Bolken.
Oregon State University; SIGA Technologies, Corvallis, OR, USA.

115. vvI7L: An Antiviral Drug Target.
D.E. Hruby, C.M. Byrd, T.C. Bolken, A.M. Mjalli, M.N. Arimilli, R.C. Andrews, R. Rothlein, T. Andrea, M. Rao, and K.L. Owens.
Oregon State University; SIGA Technologies, Corvallis, OR; TransTech Pharma, High Point, NC, USA.

116. Activity of Ether Lipid Esters of Acyclic Nucleoside Phosphonates Against Adenovirus Replication In Vitro.
C.B. Hartline, K.M. Gustin, W.B. Wan, S.L. Ciesla, J.R. Beadle, K.Y. Hostetler, and E.R. Kern.
The University of Alabama School of Medicine, Birmingharn, AL, USA; VA San Diego Healthcare System and the University of California, San Diego, La Jolla, CA, USA.

117. Activity of Different Classes of Nucleoside and Nucleotide Analogues Against Adenovirus in Cell Culture.
L. Naesens, Liesbeth Lenaerts, A. Holý, J. Balzarini, and E. De Clercq.
Rega Institute for Medical Research, K.U. Leuven, Belgium; Academy of Sciences of the Czech Republic, Czech Republic.

118. Antiviral Activity of New Triazine Nucleoside Analogues.
L. Nosach, I. Alexeeva, L. Palchikovskaya, O. Povnitsa, L. Usenko, and N. Dyachenko.
NAS of Ukraine; NASU, Kyiv, Uraine.

119. A Colorimetric Cell Culture Assay for the Identification of SARS Coronavirus Inhibitors.
E. Keyaerts, L. Vijgen, J. Neyts, F. De Clercq, J. Balzarini, and M. Van Ranst.
Rega Institue for Medical Research, K.U. Leuven, Belgium; U.Z. Leuven, Leuven, Belgium.

120. Antiviral Activity of Glycyrrhizic Acid (GL) Derivatives Against SARS-Coronavirus (SARS-CoV) and Human Cytomegalovirus (HCMV).
G. Hoever, L. Baltina, R. Kondratenko, L. Baltina Jr., G. Tolstikov, H.W. Doerr, and J. Cinatl.
Johann Wolfgang Goethe University Frankfurt, Frankfurt, Germany; Ufa Research Centre of Russian Academy of Sciences, Ufa, Russia.

121. Antiviral and Virucidal Activities of Oreganol P73-based Spice Extracts Against Human Coronavirus In Vitro.
M.K. Ijaz, Z. Chen, S.S. Raja, D.B. Suchmann, P.W. Royt, C. Ingram, J.K. Gray, and G. Paolilli.
Microbiotest, Inc., Sterling; George Mason University, Fairfax, VA, USA; University of Ottawa, Ottawa, Ont., Canada; North American Herb & Spice, Buffalo Grove, ILL, USA.

122. Mannose-Specific Plant Lectins are Potent Inhibitors of Coronavirus Infection Including the Virus Causing SARS.
J. Balzarini, L. Vijgen, F. Keyaerts, E. Van Damme, W. Peumans, E. De Clercq, H. Egberink, and M. Van Ranst.
Rega Institute for Medical Research, K.U. Leuven; U.Z. Leuven, Leuven, Belgium; RUGent, Gent, Belgium; Utrecht University, Utrecht, The Netherlands.

123. Antiviral Effect of Plant Compounds of the Alliaceae Family Against the SARS Coronavirus.
L. Vijgen, E. Keyaerts, E. Van Damme, W. Peumans, E. De Clercq, J. Balzarini, and M. Van Ranst.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; RUGent, Gent; U.Z. Leuven, Leuven, Belgium.

124. Possibilities to Inhibit the Replication of a Severe Acute Respiratory Syndrome (SARS)-Associated Coronavirus.
M. Schmidtke, C. Meier, M. Schacke, B. Helbig, V. Makarov, and P. Wutzler.
Friedrich Schiller University, Jena; University of Hamburg, Hamburg, Germany; Research Center of Antibiotics, Moscow, Russia.

125. Influenza-Inhibitory Effects of Viramidine in Cell and Animal Systems.
R.W. Sidwell, K.W. Bailey, M.-H. Wong, and D.F. Smee.
Utah State University, Logan, UT, USA.

126. Antiinfluenza Rimantadine and Novel Phenol-Contained Compounds Efficacy after Transdermal Delivery.
I. Kravchenko, A. Alexandrova, V. Lozitsky, R. Lozitskaya, V. Larionov, and N. Ovcharenko.
Odessa National University of I.I. Mechnikov, Ukraine; Ukrainian I.I. Mechnikov Research Anti-Plague Institute, Ukraine; A.V. Bogatsky Physics-Chemical Institute of NAS of Ukraine, Odessa, Ukraine.

127. Antioxidant Properties of a Plant Preparation with Anti-Influenza Virus Activity.
J. Serkedjieva, M. Sokmen, M. Angelova, and A. Sokmen.
Bulgarian Academy of Sciences, Sofia, Bulgaria; Cumhuriyet University, Sivas, Turkey.

128. Protease Inhibitors from *Streptomyces* Inhibit Influenza Virus Replication.
J. Serkedjieva, L. Angelova, and I. Ivanova.
Bulgarian Academy of Sciences; Sofia University, Sofia, Bulgaria.

129. The Idenitification of Novel Small Molecule Inhibitors of Respiratory Syncytial Virus.
J. Stables, M. Carter, D. Alber, E. Henderson, V. Dowdell, K. Kelsey, S. Keegan, B. Baxter, S. Cockerill, D. Taylor, P. Chambers, S. Tymms, K. Powell, and L. Wilson.
Arrow Therapeutics, Ltd.; Virogen, Ltd., London, UK.

130. Antiviral Terpenoids from the Chinese Medicinal Plant Schefflera Octophylla.
Y. Li, P.P.H. But, E.C.O. Vincent.
The Chinese University of Hong Kong, Hong Kong, China.

131. VP1 Sequence Analysis and Susceptibility of Laboratory Strains and Clinical Isolates of Coxsackievirus B3 (CBV3) to Pleconaril.
M. Schmidtke, E. Hammerschmidt, R. Zell, O. Riabova, V. Makarov, and P. Wutzler.
Friedrich Schiller University, Jena, Germany; Research Center of Antibiotics, Moscow, Russia.

132. Effect of Disoxaril on the Replication of Disoxaril-Sensitive and Disoxaril-Dependent Mutants of Coxsackievirus B1.
I. Nikolova and A.S. Galabov.
Bulgarian Academy of Sciences, Sofia, Bulgaria.

133. Substituted Imidazol [4,5-b]pyridin-2-ones and -thiones as Inhibitors of Coxsackie B3 Replication.
G. Pürstinger, K. Putzer, M. Widmoser, E. Padalko, E. De Clercq, and J. Neyts.
Universitat Innsbruck, Innsbruck, Austria; Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium.

134. Measurement of Neutralizing Antibodies to Interferon-β by an MxA Assay using ELISA.
Y. Gong, E. Gibbs, I. Gadawski, T. Tam, and J. Oger.
Viridae Clinical Sciences, Inc.; The University of British Columbia; MS Special Therapies, Vancouver, BC, Canada.

135. Evaluation of Bioavailability of Interferons by One-Step Real-Time RT-PCR Assay for Measurement of MxA mRNA Expression.
Y. Gong, E. Gibbs, I. Gadawski, T. Tam, and J. Oger.
Viridae Clinical Sciences, Inc.; The University of British Columbia; MS Special Therapies, Vancouver, BC, Canada.

136. Fully Size Human Antibodies Against Ebola Virus.
T. Yun, L. Shingarova, T. Batanova, and N. Tikunova.
State Research Centre of Virology and Biotechnology Vector, Koltsovo, Novasibirsk Region, Russia.

137. Protective and Immunomodulating Effect of Proteolysis Inhibitor During Experimental Rabies Infection.
A.S. Fedchuk, V.P. Lozitsky, A.A. Kravchenko, A. Yu Boschenko, I.N. Grigorasheva, and T.L. Gridina.
I.I. Mechnikov Ukrainina Research Anti-Plague Institute, Odessa, Ukraine.

138. Cloning and Sequencing of Variable Domains of Protective Mabs Against Tick-Borne Encephalitis Virus.
T.A. Batanova, L.E. Matveev, T.E. Yun, A.A. Ilyichev, and N.T. Tikunova.
State Research Centre of Virology and Biotechnology Vector, Koltsovo, Novosibirsk Region, Russia.

139. Identification and Characterization of a Potent and Specific Small Molecule Inhibitor of Human Rotavirus.
M. Kealey, L. Flay, T. Demenczuk, T. Bailey, G. Rhodes, D.C. Young, D.C. Pevear, and S. Laquerre.
ViroPharma, Inc., Exton, PA, USA.

Thursday, May 6

Oral Session VI: Hepacivirus Infections

Chairpersons: Craig Gibbs and Johan Neyts

8:30 a.m. Plenary Speaker
Stanley Lemon, University of Texas Medical Branch, Galveston, TX, USA
"Impact of Specific Antiviral Therapy on Hepatitis C Virus Suppression of Host Cell Defenses"

9:00 140. HCV NS3 Protease and NS5B Polymerase Inhibitors as Antiviral Agents.
P.L. Beaulieu.
Boehringer Ingelheim (Canada), Ltd., Laval, Que., Canada.

9:15 141. Inhibition of Heptitis C Virus RNA Replication by Benzimidazoles with High Affinity for the 5′-Untranslated Region of the Genomic RNA.
E.E. Swayze, P. Seth, E.A. Jefferson, A. Miyaji, S. Osgood, R. Ranken, S. Propp, K. Lowery, and R.H. Griffey.
Ibis Therapeutics, Carlsbad, CA, USA.

9:30 142. Nucleoside Inhibitors of HCV Replication and the Corresponding Nucleoside Triphosphate Inhibitors as Chain Terminators of HCV NS5B Polymerase.
L. Lou, C. Hancock, D. Latour, J. Pouliot, C. Roberts, J. Keicher, N. Dyatkina, R. Griffith, U. Schmitz, and E. Michelotti.
Genelabs Technologies, Inc., Redwood City, CA, USA.

9:45 143. In-Vitro and In-Vivo Evaluation of HCV Polymerase Inhibitors as Potential Drug Candidates for Treatment of Chronic Hepatitis C Infection.
E. Ilan, A. Zauberman, S. Aviel, O. Nussbaum, E. Arazi, O. Ben-Moshe, D. Slama, I. Tzionas, Y. Shoshany, S.-W. Lee, J.-J. Han, S.-J. Park, G.-H. Lee, E.-Y. Park, J.-C. Shin, J.-W. Shu, J.-W. Kim, and S. Dagan.
XTL Biopharmaceuticals, Ltd., Rehovot, Israel; B&C Biopharm; Myong Ji University, Korea.

10:00 Break

10:30 144. Susceptibility of Different Genotypes of Hepatitis C Virus to Inhibition by Nucleoside and Nonnucleoside Inhibitors.
S. Carroll, C. Bailey, M. Bosserman, C. Burlein, A. Simcoe, J. Fay, S. Ludmerer, D. Graham, R. LaFemina, O. Flores, D. Hazuda, S. Altamura, G. Migliaccio, L. Tomei, R. De Francesco, V. Summa, and D. Olsen.
Merck Research Laboratories, West Point, PA, USA; Instituto Ricerche di Biologia Molecolare, Merck Research Laboratories, Pomezia, Italy.

10:45 145. Comparison of Two Anti-HCV Clinical Candidates, VX-950 and BILN 2061, for Potency and Resistance in the HCV Replicon System and in a Novel HCV Protease Animal Model.
K. Lin, C. Lin, G. Kalkeri, S. Almquist, Y.-P. Luong, B.G. Rao, Y. Wei, C.A. Gates, R.B. Perni, and A.D. Kwong.
Vertex Pharmaceuticals, Inc., Cambridge, MA, USA.

11:00 146. Gene Expression Patterns During Anti-HCV Treatment with Interferons and Interferon Combinations in the HCV Replicon Model.
B. Korba, C. Okuse, and J. Rinaudo.
Georgetown University, Rockville, MD, USA.

11:15 147. A Novel Highly Selective Inhibitor of Pestivirus Replication That Targets the Viral RNA Dependent RNA Polymerase.
J. Paeshuyse, P. Leyssen, E. Mabery, N. Boddeker, R. Donis, I.H. Ansari, F. Koenen, P. Kerkhofs, L. Gil, C. Letellier, J. Watson, E. De Clercq, G. Pürstinger, and J. Neyts.
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Gilead Sciences, Foster City, CA, USA; University of Nebraska-Lincoln, NE, USA; Veterinary and Agrochemical Research Centre, Ukkel, Belgium; University of Innsbruck, Austria.

11:30 148. Chimeric Yellow Fever-Dengue Tetravalent Vaccine (ChimeriVax^{TM}-DEN1–4): Preclinical Safety and Efficacy in Non-human Primates.
K. Draper, C. Fournier, I. Levenbook, G. Myers, F. Guirakhoo, and T.P. Monath.
Charles River Laboratories, Sparks, NV, USA; Aventis Pasteur, Marcy-L-Etoile, France; Acambis, Inc., Cambridge, MA, USA.

11:45 149. Late Breaker Presentation

12:00 Lunch

Thursday, May 6

Oral Session VII: Late Breaker Presentations

Chairpersons: Joe Colacino and Richard Wagner

1:30 p.m. 150. PG 301029 Inhibits the HCV Surrogate BVDV Through a Novel Late Stage Mechanism of Action.
T.B. Parsley, R.W. Buckheit Jr., and T.L. Brenner.
GeneLogic, Inc., Gaithersburg, MD, USA; University of Arizona College of Pharmacy, Tucson, AZ, USA.

1:45 151. Identification of Antiviral Nucleoside Analog Metabolizing Enzymes Through Bioinformatic Data Mining.
P.J. Lorenzi, C.P. Landowski, X. Song, L.B. Townsend, J.C. Drach, and G.L. Amidon.
University of Michigan, Ann Arbor, MI, USA.

2:00 152. Antiviral Action of Interferons on Human Coronavirus.
F. Dianzani, C. Scagnolari, E. Vincenzi, F. Bellomi, M. Clementi, and G. Antonelli.
University “Campus Bio-Medico”; University “La Sapienza”, Rome; “S. Raffaele” Scientific Institute, Milan, Italy.

2:15 153. Integration of Clinical Data, Pathology, and cDNA Arrays in Influenza-Infected Pigtail Macaques: A Novel Approach for Understanding the Genetic Basis of Virulence.
C.R. Baskin, A. García-Sastre, T.M. Tumpey, H. Bielefeldt-Ohmann, V.S. Carter, and M.G. Katze.
University of Washington School of Medicine; Washington Regional Primate Research Center, Seattle, Washington; Mount Sinai School of Medicine, New York, NY, USA; U.S. Department of Agriculture, Athens, GA, USA.

2:30 154. Novel Small Molecule Inhibitors of Rhinovirus Replication that Target the HRV 2B Nonstructural Protein.
D.J. Tenney, C.P. Bergstrom, K.P. Pokomowski, S.M. Levine, D. Hernandez, S. Zhang, F. He, G. Zhai, F. McPhee, S.P. Weinheimer, J.L. Romine, and R.J. Colonno.
Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT, USA.

2:45 Late Breaker Presentation

3:00 Late Breaker Presentation

3:15 Late Breaker Presentation

3:30 Late Breaker Presentation

3:45 Late Breaker Presentation

4:00 Adjourn

Oral Session I: Retrovirus Infections

1

Nucleoside Triphosphate Mimics (P3Ms) and Their Prodrugs (P3M-Ds): A New Class of Agents with Potent Activity Against Resistant HIV-1

N. Boyle, P.D. Cook, G. Wang, T.W. Bruice, J. Leeds, J. Brooks, P. Fagen, V. Rajwanshi, M. Prhavc, T. Hurd, F. Chen

Biota, Inc., Carlsbad, CA, USA

Biota, Inc. has undertaken a broad chemical modification program focused on the triphosphate moiety (TP) of nucleoside triphosphates (NTPs) in order to circumvent the requirement for intracellular anabolism of nucleosides. Our objectives have been to discover P3M inhibitors of polymerases that resist chemical and enzymatic degradation yet, when bound in the active site, incorporate nucleoside monophosphate (NMP) analogs that potently and selectively terminate chain elongation. A subsequent objective of our drug discovery platform is the development of prodrugs of P3Ms (P3M-PDs), that enhance cellular uptake of the P3Ms. **Results:** (1) We have discovered several types of modified triphosphate moieties (TP) that, when conjugated to the eight registered nucleoside reverse transcriptase inhibitors (NRTIs) and closely related nucleosides, provide P3M-RTIs that inhibit wt and mutant HIV-1 RTs at essentially the same level as the parent NRTI-TPs. For example, the boranodifluoromethylene (BDFM) P3M of 3TC (B-558) inhibits the M184V ($3TC^R$ RT) with a K_i of 12.5 μM compared to 495 μM for 3TC. Thus, the relative change of K_i for 3TC towards the mutated RT versus wt RT is >2600-fold increase, whereas B-558 exhibits only an 80-fold increase. (2) These P3Ms exhibit stability in serum and cell extracts with half-lives of greater than 48 h. (3) The BDFM P3M of AZT-TP (B-108) has been conjugated with a number of generic prodrug moieties that enhance cell uptake. (4) P3M-PDs of AZT-TP liberate the active P3M intracellularly, which selectively and potently inhibits viral replication of wild type and resistant HIV strains to undetectable levels

2

Direct Measurement of Antiviral Nucleoside Monophosphate Delivery from Phosphoramidate Pronucleotides by Stable Isotope Labeling and LC-ESI-MS/MS

C.R. Wagner, J. Kim, T.F. Chou

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Amino acid phosphoramidates of nucleosides have been shown to be potent antiviral agents with the potential to act as nucleoside monophosphate prodrugs. To access their ability to deliver AZT-5′-monophosphate to cells, the decomposition pathway of ^{18}O labeled AZT amino acid phosphoramidates was investigated by capillaxy reverse-phase high-performance liquid chromatography and negative ion electrospray ionization mass spectrometry (LC/ESI^--MS). ^{18}O labeled (L)-AZT tryptophan phosphoramidate methyl ester (^{18}O-ATO) was synthesized with a $^{18}O/^{16}O$ relative ratio of 1.22. For CEM cells, a human T-lymphoblast leukemia cell line, incubated with ^{18}O-ATO, values of 1.55, 0.34 and 0.13 were found for the $^{18}O/^{16}O$ relative ratio of intracellular AZT-MP at time intervals of 0.5, 4 and 20 h, respectively. The decrease in labeled AZT-MP in CEM cells corresponded to a rapid increase in the amount of intracellular AZT presumably by dephosphorylation of AZT-MP. In contrast, for peripheral blood mononuclear cells (PBMCs) the $^{18}O/^{16}O$ relative ratio values of intracellular AZT-MP were 1.43, 1.06 and 0.61 for the time intervals of 0.5, 4 and 20 h, respectively. Intracellular in PBMCs was nearly undetectable for each time interval. Taken together these results are consistent with the detection of direct P–N bond cleavage by CEM cells and PBMCs. However, AZT phosphoramidates are able to more effectively deliver AZT-MP to PBMCs than to CEM cells. Although applied to phosphoramidate pronucleotides, the judicious use of ^{18}O labeling and LC/MS is a general approach that can be applied to the investigation of the intracellular fate of any pronucleotide.

Acknowledgment: Supported by contract NIH-NCI CA 889615.

3

Synergistic Anti-HIV Activity of Zinc-Finger Inhibitory Molecules Used in Combination with a Variety of Other Anti-HIV Agents

R.W. Buckheit Jr.[1], J. Russell[2], T.L. Hartman[1], J.K. Imnan[3], M. Schito[3], A. Goel[3], E. Appella[3], J.A. Turpin[4]

[1]GeneLogic Inc., Gaithersburg, MD, USA; [2]Southern Research Institute, Frederick, MD, USA; [3]National Cancer Institute, Bethesda, MD, USA; [4]The Henry M. Jackson Foundation, Rockville, MD, USA

The nucleocapsid p7 protein (NCp7) includes two retroviral zinc finger domains [$Cys(X)_2Cys(X)_4His(X)_4Cys(CCHC)$] that play a pivotal role during early and late phases of HIV-1 replication. We have chosen to pursue potential therapeutic strategies that target the NCp7 based on its broad and essential functions in virus replication and in light of its potential access to chemical inhibitors. The nucleocapsid of HIV and all other retroviruses, with the exception of the spumaretroviruses, contain one or two copies of a highly conserved Zn finger motif. Mutation in either Zn-chelating or non-chelating residues results in loss of NCp7 function and renders the virus non-infectious. Diverse sets of electrophilic compounds that react with cysteine thiolates in the NCp7 have been identified. All of these compounds are oxidizing agents that lead to ejection of chelated Zn ions, resulting in antiviral activity against all evaluated laboratory-derived and clinical strains of HIV. Our devel-

opment of inhibitory molecules has included a wide variety of chemotypes and has involved a complete evaluation of the antiviral properties of the compounds. Importantly, a significant level of antiviral synergy was observed when the compounds are used in combination with other anti-HIV agents, including attachment/fusion, reverse transcriptase and protease inhibitors and other Zn finger inhibitor chemotypes. Inhibition of HIV has been observed at similarly high levels with each of these classes of molecules. The results of our in vitro combination assays with several potent ZN-finger inhibitors will be presented.

4

Discovery of a Novel Class of Small-Molecule HIV Entry Inhibitors that Target the gp120-Binding Domain of CD4

Q. Yang[1], P. Roberts[1], W. Zhu[1], A. Stephen[2], J. Adelsberger[3], M. Currens[4], Y. Feng[5], R. Fisher[2], A. Rein[5], R. Shoemaker[4], S. Sei[1]

[1]Laboratory of Antiviral Drug Mechanisms, USA; [2]Protein Chemistry Laboratory, USA; [3]Clinical Services Program, SAIC-Frederick, USA; [4]Screening Technologies Branch, USA; [5]HIV Drug Resistance Program, NCI-Frederick, Frederick, MD, USA

HIV entry process mediated by specific interactions between viral envelope and cell-surface receptor/co-receptor presents a number of potential antiviral targets. Entry inhibitors developed to date include fusion inhibitors (targeting gp41) and chemokine receptor antagonists. Disruption of HW gp120-CD4 binding has also been attempted by various antibodies, CD4-mimicking proteins or gp120-binders, although their clinical efficacy has yet to be seen. Through cell-based antiviral mechanistic characterization of lead compounds selected from various retroviral molecular target screenings of NCI chemical library, we identified a group of antimony-containing small-molecule compounds that exerted a potent anti-HIV activity in vitro. NSC 13778 (MW 319) and its analogs blocked HIV infection in CEM-SS cells with EC_{50} (μM) of 3.84 ± 0.71 to 1.25 ± 0.38 (mean $\pm$ S.D.) and CC50 $> 200\,\mu$M. Their significant anti-HW activity was similarly demonstrated in MAGI cells. However, the activity was substantially reduced if compound addition was delayed for more than 2 h. The compounds also blocked the fusion of viral receptor-expressing MAGI cells and viral envelope-expressing HL2/3 cells. Using a competitive capture ELISA method, wherein excessive amounts of target protein were added to a fixed dose of capture ligand (solid phase) with or without test compounds, we found that NSC 13778 analogs significantly decreased the amount of gp120 bound to solid phase sCD4, but not that of sCD4 bound to solid phase gp120, indicating the compounds most likely competed with gp120 on CD4-binding. Furthermore, flow cytometric analysis showed that CEM-SS cells incubated with NSC 13778 analogs had decreased reactivity to anti-CD4 mAb known to recognize gp120-binding site as compared to untreated control. Our data suggest that these compounds inhibit HIV infection by targeting gp120-binding domain of CD4 molecules. Further efforts are warranted to explore the potential utility of these small-molecule compounds as a new class of HIV entry inhibitors.

Acknowledgment: Supported in part by NCI contract No. NO1-CO-12400.

5

HIV-1 Induced AIDS is an Allergy and the Allergen is the Shed gp120

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HIV-1/AIDS has been an unsolved problem for the last 20 years. It is now hypothesized that HIV-1, a replicating virus infection, engenders immune deficiency in patients by the same mechanism used by environmental allergens to cause immune deficiency and allergies.[1] Recent studies have revealed that the shed viral gp120 has a superantigen domain that is capable of binding to the V_H3 sequence of IgE molecules, which are bound to Fc epsilon receptor I (FcεRl) on mast cells, basophils, monocytes, and dendritic cells. The gp120 binding induces these cells to release and synthesize IL-4. Subsequently, T helper 2 cells are polarized, B cells are induced to synthesize IgE, and T helper 1 cells and CTL precursors are inactivated, all of which leads to acquired immune deficiency. Allergens were found to have superallergen domains for binding to the V_H3 sequences of IgE on hematopoietic cells, which precipitate IL-4 induced allergies. The present hypothesis implies that in addition to treatment with antivirals, HIV-1 patients should be treated with anti-allergic drugs (e.g. immune response modifiers). Moreover, it appears that effective antiviral vaccines should entail gp120 that have had their superantigen sequence deleted.

References: [1] Y. Becker, 2004. Virus Genes 28, 1–14.

6

Multiple Mutations in Human Immunodeficiency Virus-1 Integrase Confer Resistance to the Phase I/II Clinical Trial Drug S-1360

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Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium and The European TRIoH Consortium

The diketo analogue S-1360 is the first HIV-1 integrase strand transfer inhibitor (INSTI) that has entered clinical development [Billich, Curr. Opin. Investig. Drugs 4 (2003) 206–209]. We have examined the development of HIV-l resistance to S-1360 by selecting HIV-l(III_B) in the pres-

ence of increasing concentrations of the compound. The III_B/S-1360res strains selected after 30, 50 and 70 passages in the presence of S-1360 were evaluated genotypically by sequence analysis and phenotypically using the MT-4/MTT system. Multiple mutations, nine in total, emerged in the catalytic domain of integrase as a result of the selection process. They included T66I and L74M, that have both been previously associated with resistance against the diketo acid L-708,906. The progressive accumulation of mutations coincided with an increasing level of resistance of the selected strains towards S-1360. After 30, 50 and 70 passages in the presence of S-1360, III_B/S-1360res displayed a 3.8, 7.9 and >63-fold reduction in susceptibility to S-1360, respectively. Phenotypic cross-resistance to L-708,906 was observed for all selected strains. In contrast, III_B/S-1360res remained fully susceptible to V-165, an integrase binding inhibitor (INBI) belonging to the class of pyranodipyrimidines [C. Pannecouque, et al., Curr. Biol. 12 (2002) 1169–1177]. All selected strains exhibited wild-type sensitivity to inhibitors of viral entry, reverse transcriptase or protease. Recombination of the mutant *integrase* genes into wild-type background by *integrase*-Chimeric Virus Technology entirely reproduced the observed resistance profile of all the in vitro selected virus strains, confirming that the mutations found in *integrase* are indeed fully responsible for the resistance phenotype of III_B/S-1360res. In conclusion, selection of resistance to the clinical drug candidate S-1360 in cell culture, was associated with the emergence of up to nine mutations, some of which have not been previously linked to resistance to integrase inhibitors. The accumulation of nine mutations in a conserved protein suggests a complex mechanism of resistance development and may point to adaptation at the enzymatic level.

7

Ribonucleotide Reductase Inhibitors Didox and Trimidox Compared to Hydroxyurea to Inhibit Retrovirus Replication Alone or in Combination with Abacavir, ddl or Tenofovir

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Background: Ribonucleotide reductase (RR) inhibition as a strategy to impair HIV replication can function by depleting the dNTP pools required for proviral DNA synthesis as well as potentiating anti-HIV deoxynucleoside reverse transcriptase inhibitors (NRTI)s. This strategy gained credibility by the success of HU to enhance the NRTI ddl in clinical trials. HU in HIV therapy has not demonstrated single agent activity. The RR inhibitors(RRI) DX and TX have shown more antiretroviral activity than HU when used alone or with ddl in murine retroviral models. This report focuses on whether or not a guanosine (abacavir) (ABC) or an adenosine nucleotide (tenofovir) (TFV) as well as the ddl NRTI could be enhanced by a RRI. **Methods:** MAIDS infected mice were treated daily (i.p.) starting 1 week after infection with RRI alone or combined with ddl, ABC or TFV. Treatment continued for 8 weeks. Antiviral activity was assessed by spleen and lymph node size and IgG as well as proviral DNA levels. **Results:** At the non-optimum dosage used, the six-fold increase in spleen size in the ABC and ddl studies or the 4.7-fold increase in the TFV experiment were nearly completely prevented by DX + ABC or DX + ddl, and 80% by DX + TFV. HU + ABC, HU + ddl and HU + TFV impaired the spleen size increase by about 80, 70 and 50%, respectively. ABC, ddl or TFV alone had only a small effect of about 20% or less. The proviral DNA levels correlated with the spleen data. DX + ABC, DX + ddl or DX + TFV almost completely prevented proviral DNA synthesis. HU + NRTI combinations were less effective, ranging from 50 to 80%. TX + NRTI combinations were generally between DX and HU values. **Conclusion:** These data support the concept of using RR inhibitors for the treatment of retrovirus infection and adds two new NRTI antiviral agents, ABC and TFV, in addition to ddl, that can be enhanced by PRIs, in particular the novel RRI DX.

Oral Session II: Hepadnaviruses, Retroviruses, West Nile Virus

8

Generation of Novel Stable Cell Lines for Cross-Resistance Testing of Anti-HBV Compounds by Real-time PCR in a 96 Well Format

W.E. Delaney IV, H. Yang, A. Sabogal, X. Qi, M. Miller, S. Xiong

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Background: Susceptibility testing of anti-HBV agents is typically carried out by transient transfection of HBV DNA into hepatoma cells followed by Southern hybridization. This method is reliable, but labor- and time-intensive. Furthermore, sensitivity is limited for mutants with reduced in vitro replication fitness. **Aims:** (1) To generate stable cell lines expressing high levels of wild-type (wt) and lamivudine-resistant (LAM-R) HBV. (2) To determine cross-resistance profiles of developmental anti-HBV drugs in an efficient assay format. **Methods:** A plasmid encoding a wt HBV genome driven by the CMV promoter and a *neo* selection marker was used to generate four LAM-R HBV strains by site-directed mutagenesis. LAM-R mutants were: rtM204I, rtL180M + M204V, rtLI80M + M2041, and rtV173L + LI80M + M204V. Following transfection of HepG2 cells, stable cell lines were selected using G418. For IC_{50} determination, cells were seeded in 96 well plates and treated with compounds for 1 week. HBV DNA was quantified in cytoplasmic extracts by real-time PCR using an ABI 7900HT and primers/probes described previously [Weinberger, J. Virol. Meth. 85, 75–82]. **Results:** Cell lines expressing high levels of HBV DNA were isolated for wt and each of the four LAM-R mutants. Using a 96 well real-time PCR assay, IC_{50}s of anti-HBV drugs could be efficiently and reproducibly determined. All L-nucleosides (LAM, ETC, L-FMAU, L-dC, L-dA, and L-dT) showed significant cross resistance in cell lines expressing LAM-R HBV. Acyclic phosphonate nucleotides (adefovir, tenofovir, and MCC-478) retained efficacy in cell lines expressing LAM-R HBV. IC_{50}s generated by real-time PCR showed an excellent correlation with previous values generated by Southern hybridization. **Conclusions:** Novel stably transfected cell lines that express high levels of all major clinical patterns of LAM-resistant HBV have been established. These cell lines allow the efficient determination of antiviral activity against wild-type and LAM-R HBV in 96 well format and will be useful for further drug discovery and development efforts.

9

Long Term Incidence and the Molecular Basis of Hepatitis B Resistance to Adefovir Dipivoxil (ADV)

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Lamivudine (LAM) resistance occurs in 42 and 70% of chronic hepatitis B patients after 2 and 4 years, respectively. The ADV resistance mutations rtN236T and rtAl8lV were observed in 2% of patients at 96 weeks. **Aims:** (1) Determine incidence of ADV resistance over 144 weeks. (2) Characterize the rtN236T mutation and study mechanisms of rtN236T-mediated resistance. **Methods:** Resistance surveillance included 629, 293, and 167 patients who received ADV through 48, 96, and 144 weeks, respectively. HBV reverse transcriptase (RT) was sequenced for all samples with detectable HBV DNA ($\geq 3 \log_{10}$ c/ml) by PCR. Cumulative probability of resistance was calculated using the Life Table method. Drug susceptibility and viral replication fitness were determined by transient transfection and quantification of intracellular HBV DNA. A computer model of HBV RT based on HIV-1 and MuLV RT structures was used to study mechanisms of rtN236T-mediated resistance. **Results:** The cumulative incidences of ADV resistance (rtN236T and rtAl8lV) at weeks 48, 96, and 144 were 0, 2, and 3.9%, respectively. In vitro analysis of rtN236T mutant HBV demonstrated a 9.6-fold decrease in adefovir IC_{50}. Susceptibilities to tenofovir and MCC-478 were reduced by 4.2- and 8.6-fold, respectively. The rtN236T mutant remained sensitive to LAM, L-dT, and entecavir with IC_{50} changes $<$2.4-fold. The rtN236T mutation resulted in $>$60% reduction in replication in vitro. Structure modeling suggests that the side chain of rt236T may have a more favorable interaction with the γ-phosphate of dATP compared to adefovir diphosphate, thus providing for selectivity against adefovir diphosphate versus the natural substrate. **Conclusions:** ADV resistance emerged at a low frequency with a cumulative probability of 3.9% after 144 weeks of ADV therapy in chronic hepatitis B patients. The rtN236T mutation shows full susceptibility to LAM in vitro and in vivo.

10

Novel 8-Substituted Dipyridodiazepinone Inhibitors with Broad-Spectrum Activity Against NNRTI-Resistant HIV-1

C. Yoakim, P.R. Bonneau, R. Deziel, L. Doyon, J. Duan, I. Guse, B. Haché, S. Landry, E. Malenfant, J. Naud, W.W. Ogilvie, J.A. O'Meara, R. Plante, B. Simoneau, B. Thavonekham, M. Bös, M.G. Cordingley

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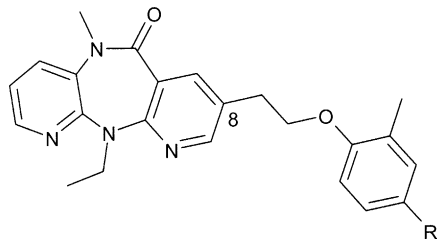
HIV-1 reverse transcriptase is a key target for the inhibition of viral replication. However, treatment with regimens containing non-nucleoside reverse transcriptase inhibitors (NNRTI) often lead to resistance due to drug-specific mutations, leaving patients with no further NNRTI options. Advanced 8-substituted dipyridodiazepinone derivatives were used as a starting point for the identification of new inhibitors with a broader antiviral profile and promising pharmacokinetic parameters. The cellular activity, biopharmaceutical and pharmacokinetic profiles of these novel analogues will be described.



11

Specific Killing of HIV Infected Cells by Cyclin Dependent Kinase Inhibitors

J. Hesselgesser, C.S. Gibbs, R. Shibata

Gilead, Foster City, CA, USA

Latent reservoirs in HIV infected individuals represent a continuing concern for physicians and patients under HAART therapy. During therapy the number of HIV infected cells declines quickly due to the short life span of activated/infected T-lymphocytes, killing by cytotoxic T cells, and the intrinsic cytotoxicity of HIV. However, even after several years of successful HAART numerous infected cells still remain. These are mostly memory T lymphocytes, carrying an integrated viral genome and expressing very low levels of viral proteins (latent reservoir). Since HIV infection affects normal cellular physiology, infected cells could be more vulnerable than uninfected cells to inhibitors of specific cellular enzymes. In support of this premise, several compounds of the cyclin dependent kinase (CDK) inhibitor class have been reported to selectively kill HIV-infected cells. Our goal was to explore a new class of anti-HIV compounds that selectively kill HIV-infected cells. Such drugs could reduce the size of the latent reservoir when used in combination with HAART. We tested over 30 CDK inhibitors and structurally related molecules simultaneously against chronically infected and uninfected CEM T cells. Cell killing was measured by XTT assay and a selectivity ratio between infected and uninfected cells was calculated. Several CDK inhibitors including, two purine analogs, one pyrazine derivative, and one paullone derivative, were 10- to 80-fold more toxic to infected cells than to uninfected cells. Six compounds showed moderate selectivity (4–10-fold), eight compounds showed poor selectivity (0.5–4-fold) and several compounds were not toxic in both cells up to 200 μM. Some CDK inhibitors that were not effective alone showed selectivity when used in combination, suggesting that inhibition of more than one CDK may be required for selective killing of HIV-infected cells. The most selective compounds also showed anti-HIV activity in primary T cells and macrophages. Collectively the data suggest that targeting of specific CDKs may represent a novel therapy for the treatment of chronic HIV infection.

12

HIV-1 Resistance to Binding/Fusion Inhibitors

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The replicative cycle of HIV can be interrupted at several stages. The reverse transcriptase and protease enzymes and the gp41-dependent fusion step are the targets of approved antiretroviral agents. However, a number of compounds are being developed that are targeted at earlier stages of infection, namely HIV adsorption (binding) to the host cells and virus–cell fusion.

Drug-resistant virus strains recovered from sequential passaging of HIV-1 in the presence of binding or fusion inhibitors showed multiple mutations in the *env* gene. These mutations confer specific drug-resistance but may also generate profound changes in the *env* gene that may alter the pathogenicity of the infecting virus. Recombinant virus, containing the envelope from a number of HIV isolates with differential replication capacity showed that gp120/gp41 play an essential role in HIV fitness. Thus, mutations in the envelope gene that confer resistance, alter the replication capacity of drug-resistant virus and may also push HIV to expand its cellular tropism.

Selection of virus-resistance to polyanionic compounds may induce changes in gp120 that reduce the virus capacity to infect different cell lines. Furthermore, selective pressure may induce changes that alter sensitivity to gp41-dependent fusion inhibitors such as T-20 or C34 suggesting that polyanionic compounds indeed have an effect on gp41-dependent fusion.

Finally, we have recently found a previously unrecognized mode of transmission of HIV-1 particles from persistently

infected to uninfected CD4+ cells that appears to be mediated by a macropinocytic mechanism that is independent of the virus-fusion process. Cell-to-cell virus transmission from infected to uninfected cells occurred in the presence of HIV binding inhibitors, HIV coreceptor inhibitors and gp41-dependent inhibitors but could be blocked by a CD4 antibody (Leu3a). HIV may then use CD4 T-cells as virion reservoirs and itinerant carriers that contribute to the spread of infection.

13

Delivery of Anti-Viral Immunoglobulins into the CNS for Treatment of Encephalitic Viruses

A.L. Olsen, J.D. Morrey

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A formidable concern for treating WNV infection is that once a patient exhibits symptoms sufficient to seek medical care, there may already be neurological involvement and may miss opportunities for effective treatment. Only supportive care is currently available for treatment of WNV. A handful of treatments have shown some efficacy in treating WNV infection in animal models. When administered prior to, or during the early stages of WNV infection before the virus has entered the brain, agents such as interferons interferon inducers, and immunoglobulin with anti-West Nile virus activity have been shown to reduce or prevent mortality. No treatment has proven effective once clinical signs of neurological involvement appear. Achieving therapeutic levels of anti-viral compounds in the central nervous system (CNS) are difficult because most anti-viral compounds cross the blood–brain barrier very poorly. A potential technique for overcoming this problem is to administer therapeutic agents directly into the CNS. A cannula was placed precisely into the left lateral ventricle of the brain of a mouse using a stereotaxic instrument for delivery of therapeutic compounds. The cannula was connected to an Alzet^{TM} osmotic pump, which allowed for constant infusion of compounds into the CNS. Human immunoglobulin was administered intraventricularly to mice at doses of 50, 5, and 0.5 μg/mouse/h. No overt toxicity was detected.

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Evidence for Transplacental Infection of Rodent Fetuses with West Nile Virus

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The CDC has reported one case of a human fetus being putatively infected with WNV from its infected mother. Our objective was to determine if rodent fetuses can be infected when pregnant dams are challenged with WNV at different times of gestation. Timed-pregnant golden Syrian hamsters were infected subcutaneously (s.c.) with 10^3 50% cell-culture infectious doses ($CCID_{50}$) on day 9 of gestation. Some WNV-infected dams delivered neonates prematurely by 1–2 days, while other dams reabsorbed their fetuses. One-day-old neonates, born to infected dams at 9 days post-maternal injection (dpmi), had an average organ titer of $10^{6.0}$ $CCID_{50}$. Timed-pregnant mice were injected s.c. with 10^4 $CCID_{50}$ on day 12 of gestation. Maternal and fetal samples were obtained at 3–5 dpmi. Virus was detected in the placenta 3 dpmi at an average titer of $CCID_{50}$. Placental viral titers increased in a time-dependent fashion, i.e. to $10^{3.8}$ $CCID_{50}$ at 4 dpmi and $10^{4.7}$ $CCID_{50}$ at 5 dpmi. Virus titers of pooled fetal organ tissues were detectable 5 dpmi with an average of $<10^{1.9}$ $CCID_{50}$. Neonates from 2 litters born to infected dams were placed with uninfected foster females 6 days after birth and were raised to weanling age. One of the foster females, nursing pups born to a WNV-injected dam, (died 6 days after the transfer of the pups and yielded a WNV-brain titer of $10^{6.8}$ $CCID_{50}$. The pups born to WNV-injected females had detectable WNV in their brains as determined by qRT-PCR. These data suggest that WNV can infect rodent fetuses in utero.

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Rapid Development of an Antisense Phosphorodiamidate Morpholino Oligomer for the Treatment of West Nile Virus

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A Phoshorodiamidate Morpholino Oligomer (PMO) was designed to inhibit the translation of the single polyprotein in West Nile Virus (WNV) referred to as AVI-4020. The efficiency for inhibition of translation was measured in an in vitro translation assay in which the WNV translation start site was fused to luciferase. Less than 20% cytotoxicity was observed with up to 50 μM PMO in Vero cells exposed for 24 h. Cell Culture studies indicate 20 μM AVI-4020 is very active against whole virus in cell culture with 2.5–4-log reduction in viral titer observed 24 h after cell treatment. Studies in the suckling mouse model revealed a nearly one log reduction in WNV titer in the mouse brain with AVI-4020 indicating the PMO may cross the blood–brain barrier. Studies were conducted in healthy rats and confirm AVI-4020 enters

the cerebrospinal fluid within the first 6 h post-intravenous injection. A collection of WNV infected Humbolt penguins at the Milwaukee County Zoo resulted in loss of body weight and ultimately nearly all infected penguins died. AVI-4020 was administered to 3 WNV infected penguins which resulted in stabilized body weights and a significant life sparing effect. These studies led to a human clinical trial in which nine infected patients with WNV encephalitis were treated with 30 mg AVI-4020 per day for 5 days. One placebo patient was also enrolled. There were no drug related adverse events. The potential for reduction in clinical signs is currently being evaluated. The span of time from initiation of this project to human clinical trial was approximately 10 months. The rapid development of therapeutic agents for other flaviviruses and other single-stranded RNA viruses appears to be feasible.

Poster Session I: Retroviruses, Hepatitis Virus Infections

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Synthesis of Novel CADA Compounds and Their Anti-HIV and CD4 Down-Modulation Activities

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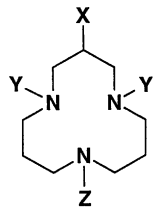
CADA (9-benzyl-3-methylene-1,5-di-*p*-toluenesulfonyl-1,5, 9-triazacyclododecane) specifically down-modulates the CD4 receptor on the surface of lymphocytes and monocytes/macrophages, the principal host cells utilized by HIV for replication. Structural modifications of CADA were made to increase potency, reduce cytotoxicity, and improve physical properties. We report the anti-HIV and CD4 down-modulation activities of side-arm analogs (Y is replaced by different sulfonamide groups) and tail analogs (Z is replaced by various hydrophobic and heterocyclic groups. Several head group analogs were synthesized which involved introduction of some good leaving/polar groups (X). Some of these head groups can regenerate the double bond of CADA by elimination reactions, potentially producing water-soluble pro-drugs. An alternative approach for the synthesis of unsymmetrical CADA analogs is being studied involving cyclization of 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (TBD) with various linkers to form a tricyclic guanidinium intermediate. This approach enables the selective synthesis of unsymmetrical side-arm analogs. The CD4 down-modulation activities of CADA analogs are summarized, and a three-dimensional model based on structure–activity relationships is also presented. Current studies are directed at pinpointing the molecular target for CADA compounds.



CADA

(X = = CH_2, Y = Ts, Z = Bn)

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Aminoglycoside-Arginine Conjugates: Chemical Barriers of HIV-1 Entry

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We have designed and synthesized aminoglycoside-arginine conjugates (AACs) as anti-HIV-1 agents.[1–3] AACs inhibit HIV-1 replication. Although designed as Tat antagonists, they also compete with monoclonal antibody binding to CXCR4, and with SDF-1α and HIV-l gp120 cellular uptake, indicating that they interfere with initial steps of HIV-1 infection. The core and the number of arginines attached to a specific aminoglycoside are important for their anti-HIV potency.[3] The most potent anti-HIV-1 AAC is the hexa-arginine neomycin B conjugate (NeoR6). NeoR6 resistant isolates have the following mutations in the surface glycoprotein gp120: 1249T in the C3 region, S283L in the V4 region, and Q306L in the C4 region; and in the transmembrane glycoprotein gp4l: S640R and F645Y in the 'heptad repeat' 2 (HR2).[4] This strongly suggest that NeoR6 obstruct HIV-1 replication by interfering with the fusion step, dependant of both conformational changes in gp120 following CD4 and CXCR4 interaction, as well as by conformational changes in gp41 induced by HR1 and HR2 interaction. One mutation in the gp120 C4 region associated with a change of nonpolar (Q) to polar (K) amino acid residues. Similarly, two positions in the HR2 of gp4l are associated with changes of nonpolar (S and F) to polar (R and Y) amino acid residues. Nonpolar residues appear to be important for the antiviral activity of NeoR6. Although the V3 domain of gp120 was found to be necessary for interaction with the HIV-I chemokine coreceptors, other domains of gp120 were also found to play a role in the interaction of gp120 with CCRS. The role of the V3 domain in the interaction of gp120 with CXCR4 has not been directly demonstrated; variable domains, such as V1/V2 and V3 of gp120, can both contribute to the interaction with CXCR4 coreceptor. Therefore, mutations in NeoR6 resistant isolates may confer resistance by allowing the interaction of gp120 with CXCR4 in such a way that NeoR6 interference would be minimized. The mechanism of the dual functions of NeoR6, inhibiting HIV-1 entry by binding CXCR4 coreceptor and inhibiting the fusion process is being further investigated. The AACs may thus represent a novel family of fusion inhibitors.

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Synthetic Polymeric Antagonists for Receptors of HIV-1 Entry into Human Cells

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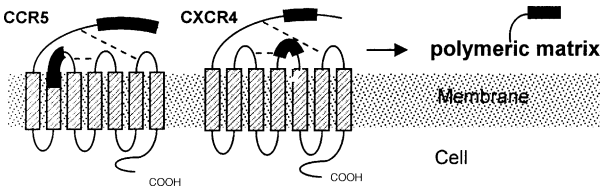
A penetration of human immunodeficiency viruses type I (**HIV-1**) into permissive human cells is driven by the viral env proteins (gp120 and gp41) due to specific interaction with the cellular receptor CD4 (adsorption gp120-CD4) and α-chemokine receptors **CCR5** and/or **CXCR4** (co-adsorption CD4-gp120-CCR5/CXCR4 and gp41 activation for fusion). The analysis of the modern data of the **CCR5** and **CXCR4** macromolecular structure–functional organization [Biotechnol. Russia 2002 (4) 11–23; Biotechnol. Russia 2003 (4)] allows to choose the submolecular structures which play a critical role as molecular mediators for **HIV-1** entry into cells. Number of these sites were reconstructed to synthetic peptides (12–18 aa), modified by N-term. Lys that includes tetramethylenic spacer terminated by amino group, which were used for chemical incorporation into macromolecular matrixes of synthetic analogs of succinic acid (imitator of an extracellular negative charge which can recompense a desulfating of Tyr from the peptides).



Unlike the initial peptides and unmodified polymeric matrixes the chemically cooperated conjugates exhibit in vitro high anti-**HIV-1** activity (IC_{50}/CC_{50} up to 1000) against the various **HIV-1** strains, including **AZT**-resistant isolates. In contrast to the native cellular receptors them synthetic polymeric analogs are well soluble in water media, not fixed by membrane and, as result, they are more mobile agents for a competitive attack and preventive inactivating of virions.

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RDS1643, a Novel Diketo Acid which Selectively Inhibits the HIV-1 Multiplication in Cell-Based Assays and the Ribonuclease H Activity in Enzyme Assays

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The HIV-1 reverse transcriptase (RT) is a multifunctional enzyme which displays DNA polymerase activity on both RNA and DNA templates and a degradative activity, termed ribonuclease H (RNase H), that hydrolyzes the RNA component of the heteroduplex RNA:DNA replication intermediate. While both activities of the RT are required for viral replication, until now only the polymerase functions have been widely explored as drug target. Recently, a diketo acid derivative has been shown to inhibit the in vitro RNase H activity, although being ineffective on the viral replication in cell-based assays [Shaw-Reid et al., J. Biol. Chem. (2003)]. We have identified a novel diketo acid derivative, RDS1643, which inhibits the HIV-1 RNase H activity in vitro while it was ineffective on the RNA-dependent DNA polymerase (RDDP) activity of the H1V-1 RT and on the RNase H activities displayed by AMV and *E. coli*. Time of addition studies revealed that the compound is active in enzyme assays independently on the order of its addition to the reaction mixture. Kinetics of RDS1643 inhibition of the polymerase-independent RNase H activity showed a non-competitive pattern and a K_I value of 17 μM. When RDS1643 was combined with non-nucleoside RT inhibitors (NNRTI), such as efavirenz and nevirapine, and was evaluated in enzymatic assays against the RDDP or the RNase H activities, the new diketo acid did not affect the anti-RDDP activity of the NNRTIs and, vice versa, the NNRTIs did not affect the RDS1643-mediated inhibition of the RNase H. When assayed on the viral replication in cell-based assays, RDS1643 inhibited HIV-1_{IIIB} strain with an EC_{50} of 14 μM. Similar results were obtained against the Y181C and Y181C/K1O3N HIV-1 mutants.

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4′-Ethynyl-d4T, a Novel Nucleoside Inhibitor of HIV-1 with Unique Properties

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In the search for novel nucleoside analogs active against HIV-1 replication, 2′,3′-didehydro-3′-deoxy-4′-ethynylthy-

midine (4′-ethynyl-d4T) was found to be a potent and selective inhibitor of HIV-1 replication in cell cultures. The EC_{50}s of 4′-ethynyl-d4T for HIV-1 replication were 0.070 and 0.20 in MT-4 and MT-2 cells, respectively. The compound did not affect the proliferation and viability of these cells at concentrations up to 100 μM (CC_{50} > 100 μM). On the other hand, the EC_{50} and CC_{50} of stavudine (d4T) were 0.31 and 79 μM in MT-4 cells, respectively, indicating that 4′-ethynyl-d4T is more potent and less toxic inhibitor of HIV-1. The anti-HIV-1 activity of 4′-ethynyl-d4T was also examined for a variety of reverse transcriptase (RT)-resistant mutants. Of particular interest was that the compound equally inhibited a wild-type strain and a multinucleoside-resistant mutant containing A62V, V75I, F77L, F116Y, and Q151M. The EC_{50}s for the wild-type and the mutant were 1.5 and 1.0 μM in MAGI-CCR5 cells, respectively. In contrast, the zidovudine (AZT), d4T, didanosine (ddI), and lamivudine (3TC) were 380-, 8.5-, 14-, and 4.4-fold less inhibitory to the mutant, as compared to the wild-type. Non-nucleoside RT inhibitor-resistant mutants (K103N or Y181C) were also as susceptible as the wild-type to the compound. However, 4′-ethynyl-d4T appeared to be slightly less active against an AZT-resistant mutant (D67N, K7OR, T215F, and K219Q) and a 3TC-resistant mutant (M184V). These results suggest that 4′-ethynyl-d4T is a novel nucleoside HIV-1 inhibitor with unique properties.

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Susceptibility of HIV-2, SIV and SHIV to Various Anti-HIV-1 Compounds: Implications for Treatment and Postexposure Prophylaxis

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Limited information is available on the activity of antiretroviral drugs against human immunodeficiency virus type 2 (HIV-2) and simian immunodeficiency (SIV) strains to guide their use in treatment or prophylaxis of such viral infections. We evaluated the antiviral activity of 16 approved drugs and 1 experimental drug, AMD3100, against two wild-type HIV-2 (ROD and EHO) isolates, two strains of SIV (mac251 and B670), and two strains of simian-human immunodeficiency virus (SHIV) that contain the reverse transcriptase (RTSHIV) or envelope glycoprotein (SHIV89.6) of human immunodeficiency virus type 1 (HIV-1) in a SIV (mac239) background. The nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine, lamivudine, stavudine, didanosine, zalcitabine, and abacavir as well as the nucleotide RT inhibitor tenofovir retained full activity against all six viruses except for SIV and SHIV89.6 that showed low-level resistance to didanosine. The protease inhibitors (PIs) ritonavir, indinavir, saquinavir, and nelfinavir were found to be active against some HIV-2 or SIV strains. However, a significant reduction in susceptibility was seen with indinavir against SHIV89.6 (3.3-fold), and with amprenavir against HIV-2 (ROD) (8.8-fold). All viruses except for RTSHIV showed a >200-fold decrease in susceptibility for the non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine and efavirenz, indicating high-level resistance. AMD3100, a CXCR4 antagonist, was active against HIV-2 and SHIV89.6, but was inactive against SIV strains. In contrast, enfuvirtide (T20) was active against SHIV89.6 but had reduced inhibitory activity against both HIV-2 and SIV strains predicting little therapeutic value against these viruses. These findings support the use of NRTIs, tenofovir, but not NNRTIs, in the treatment and/or postexposure prophylaxis of HIV-2, SIV and SHIV infections; they also point to the usefulness of AMD3100 in the therapy/prophylaxis of HIV-2 and SHIV89.6 infections.

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Inhibition of HIV-1 Replication Using a Single Transcriptional Unit that Expresses Decoy and siRNAs in Mammalian Cells

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The search for an effective antidote against HIV-1 infection had evolved many strategies to mediate viral intervention in the HIV target cells. These strategies include gene therapy applications, such as intracellular expressed antisense RNAs, decoy RNAs, and RNA interference (RNAi) to mediate degradation of viral messenger RNA (vmRNA) in the infected cells. In this study, we employed the combined decoy TAR and the *vif*-siRNAs, which were simultaneously expressed as separate anti-genes in the cells. We evaluated the inhibitory efficacies of this combined HIV-1 anti-gene expression vectors under the control of the human U6 promoter. The intracellular steady-state mRNA expression of the *vif*-TAR and its controls (*vif*-siRNA, and *vif*-siRNA scramble, decoy TAR, and decoy TAR-M 31–34 with no bulge) were established to be high by Northern blotting analysis. Results of HIV-1 *gag* p24 antigen production, reporter gene (EGFP) expression, and the quantitative expression of the viral mRNA as markers for inhibition indicated down-regulation of viral replication in the Hela $CD4^+$ cells. These data therefore suggest that target-

ing HIV-1 with simultaneous intracellular expressed decoy TAR and *vif*-siRNAs could lead to an effective gene therapy application for the control and management of HIV-AIDS.

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Inhibition of Human Immunodeficiency Virus type 1 via HIV-1-dependent anti-gene expression system

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We previously demonstrated the function of an HIV-1-dependent ribozyme expression vector, with which the site-specific excision of loxP sequences can be achieved by using the Cre-loxP system (ON/OFF) as a molecular switch in an acute HIV-1 infection. However, this expression system also revealed the lower, non-specific expression of the anti-HIV-1 ribozyme in the absence of *tat*. To circumvent this problem, we used the more efficient HIV-1-dependent Cre recombinase gene expression vector, encoding the LTR-*gag-p17*. Furthermore, we constructed the ploxP-Rz-U5 and pLT-*gag-p17*-Cre plasmids, and also combined them into a single vector, pLTR-*gag-p17*-Cre/loxP-Rz-U5. The resultant simultaneous expression of the Cre protein and the homologous recombination of the two loxP sequences induced a high level of HIV-1 replication inhibition (95%). To further enhance its efficacy for human gene therapy application, we incorporated the EBNA/OriP genes into the Cre/loxP ribozyme system. This resulted in the effective replication of the recombined vector in HeLa $CD4^+$ cells. Therefore, the ribozyme expression persisted in the cells for about 1 month. These data imply that targeting the HIV-1 genes with this novel vector which mediates persistent HIV-1-dependent ribozyme expression would be a useful tool for HIV-1 gene therapy applications.

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Optimization of Unique Thioesters as Antiviral Agents that Target HIV-1 Nucleocapsid Protein (NCp7)

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The highly conserved p7 nucleocapsid protein (NCp7) of HIV-1 is a target for the development of new antiviral agents based on its broad range of function in virus replication and the ability of inhibitors to selectively interact with the fingers and eject the coordinated zinc, inhibiting virus replication and rendering the virus non-infectious. The highly conserved NCp7 protein of HIV contains two copies of the zinc finger motif $Cys(X)_2Cys(X)_4His(X)_4Cys$ (CCHC). NCp7 plays pivotal roles during both early and late phases of HIV-1 replication, being required for the functioning of the reverse transcriptase, integrase and protease, as well as the selection and packaging of the RNA genome into maturing virions. Mutations in the Zn chelating and/or non-chelating residues have been shown to result in loss of NCp7-mediated functions, rendering the virus non-infectious. Thus, the central role of the NCp7 protein and the absolute requirement for intact NCp7 protein and Zn fingers during HIV replication makes this protein an attractive target for drug development. Diverse sets of electrophilic compounds that react with cysteine thiolates in the NCp7 protein or NCp7 protein precursors ($p55^{gag}$ and $pr160^{gag\text{-}pol}$) have been identified. Although different in chemical composition all lead molecules lead to the ejection of Zn(II) ions bound within the structural Zn finger motifs of the NC protein. As a result of our efforts to increase solubility, stability and potency we have recently identified a series of less hydrophilic, uncharged *S*-acyl-2-mercaptobenzamide thioester derivatives. In order to maximize the potential to identify the optimal compound configuration, we developed a combinatorial chemistry approach to explore three spaces of the thioester chemotype. Using this model as a skeleton for synthetic variation allowed the use of substituents, which could potentially modify the reactivity of the thioester bond through electronic influences and steric hindrance. We tested the hypothesis that by increasing stability of the thioester linkage we would enhance the potency of the thioester chemotype and achieve sub-micromolar inhibitory efficacy.

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Anti-HIV Activity Profile of a Novel CCR5 Inhibitor, AMD887, in Combination with the CXCR4 Inhibitor AMD070

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The antiviral efficacy of several CCR5 antagonists (e.g. SCH-C, UK427,857) (against R5 viruses) and a CXCR4 antagonist (e.g. AMD3100) (against X4 viruses) was demonstrated in clinical Phase II studies. Here, we evaluated the in vitro anti-HIV activity of AMD887, a novel CCR5 antagonist, and of AMD070, a recently described orally bioavailable CXCR4 antagonist. AMD887 (alone and in combination with AMD070) was examined for its activity against a wide variety of HIV-1 laboratory strains

(R5, X4, R5/X4) and primary clinical isolates in different T-cell lines, cells expressing CD4 and CXCR4 or CCR5, and PBMCs. Chemokine (RANTES and SDF-1) binding and internalization, chemokine-induced Ca^{2+} signaling and chemotaxis assays were performed to demonstrate the specific interaction of the compounds with their respective chemokine receptors. AMD887 was found to act as a potent CCR5 antagonist that strongly inhibited virus infectivity at a 50% effective concentration (EC_{50}) of 1–10 nM. The compound inhibited the replication of different clades of R5 HIV in PBMCs and cells expressing CCR5. AMD887 (as also described for SCH-C and UK427,857) had no activity against X4 or R5/X4 HIV-1 isolates in PBMCs. However, these X4 and R5/X4 HIV variants were prevented from infecting PBMCs in the presence of AMD070. When both compounds, AMD887 and AMD070, were combined, no viral replication with any of the X4, R5 or R5/X4 HIV-1 strains used was measured in PBMCs. Their anti-HIV potency correlated closely with their potency in inhibiting chemokine binding, chemokine-induced internalization, chemokine-induced Ca^{2+} signaling and chemotaxis. AMD887 and AMD070 did not interact with any chemokine receptor other than CCR5 and CXCR4, respectively, examined to date. Both compounds (separate and in combination) hold great promise as candidate anti-HIV drugs.

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Cyclic Dinucleotides as Potential Inhibitors of the 3′-Processing Step of HIV-1 DNA Integration

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The molecular mechanism of integration of HIV DNA into the host cell genome appears to occur by an ordered sequence of DNA tailoring (3′-processing) and coupling (integration) reactions. Prior to the initiation of integration, there is recognition of viral DNA by the integrase. HIV integrase recognizes specific sequences in the LTRs of viral DNA. In the first step of integration (3′-processing), there is specific endonuclease activity that removes two nucleotides from each 3′-end of double helical viral DNA to produce new CAOH-3′ termini. For this initial 3′-processing step, integrase activates the phosphodiester bond towards cleavage. The resulting endonuclease activity apparently eliminates a cyclic dinucleotide (e.g. 3′,5′-cyclic pGpT) as well as a linear dinucleotide of the same sequence. In previous studies, we have shown that linear dinucleotide 5′-phosphates representing terminal dinucleotides of processed HIV viral DNA show inhibitory activity toward HIV integrase. In order to study whether another product of the 3′-processing step, i.e. a cyclic dinucleotide, could also be an inhibitor of HW integrase, we have investigated specific cyclic dinucleotides. This paper will report on the synthesis, structural and conformational studies, nuclease enzymology and HIV integrase inhibition studies of these compounds.

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Activity Assays of HIV-1 Integrase Using Microtiter Plate Technology

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Integrase (IN) mediates the integration of the retroviral genome into host chromosome. The activity of IN can be reconstituted in vitro with short DNA oligonucleotides, which mimic the viral DNA end and purified recombinant IN. We have developed a microtiter plate assay for HIV-1 IN using 5′-biotin-labeled substrate DNA and 3′-digoxygenin-labeled target DNAs. The strand transfer products are captured on a streptavidin-coated microtiter plate. The denaturation of the strand transfer products by 30 mM NaOH removes nonspecific binding of labeled substrates and IN and thus reduces the background reading. The positive reactions had OD values in between 0.5 and 0.6 compared to the background readings of 0.03–0.04. The optimum metal required for this assay was found to be 3 mM Mn^{2+}. This assay is extremely useful for studying the reaction mechanism of IN, its interaction with other proteins and enzymes, and for rapid screening of antiviral drugs. We will present work using this assay in each of these areas. Finally, we will present the transfer of this assay to a high-throughput format for screening of antiviral drugs.

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Inhibition of Late Stage Events in HIV Replication: Therapeutic Compounds with Effects on Viral RNA Synthesis

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The effective use of highly active antiretroviral therapy results in the near complete suppression of virus production in infected individuals, but does not yield suppression of low level virus production in cells in sanctuary sites and does not result in the elimination of infection. Thus, the continued search for new antiretroviral agents with unique and different mechanisms of HIV inhibition remains critical, and compounds that can reduce the level of virus production from cells already infected with HIV, as opposed to preventing de novo infection, would be of great benefit. We have discovered and evaluated a series of compounds that sup-

press HIV replication in cells which are chronically infected with HIV and which constitutively produce HIV. These compounds were only identified in screening programs specifically designed to detect inhibitors of ongoing virus replication and were variably active in traditional anti-HIV assays which monitor inhibition of acute infection. Range of action studies demonstrated the ability of the compounds to inhibit the replication of diverse strains of HIV from chronically infected cells, including protease inhibitor-resistant strains. The compounds interacted in an additive fashion with all other tested anti-HIV agents in both acute and chronic in vitro antiviral assays and resistance to the compounds was not detected even after 3 years of passage of virus in the presence of the compounds. Mechanistic studies demonstrated the specific antiviral effect of the compound relative to host cell toxicity and have defined a mechanism of action for the compounds similar to that expected for a Rev inhibitor. The compounds yield a reduction in the quantity of singly spliced and unspliced HIV RNA transcripts and an increase in the quantity of multiply spliced RNA species. The antiviral profile of these compounds and their clinical potential will be presented.

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Intracellular Neutralization of HIV-1 Replication by Immunoglobulin A

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Human immunodeficiency virus (HIV) is primarily transmitted through cervicogenital and rectal mucosal surfaces. Possible HIV entry can be via direct infection of submucosal target cells, transmucosal transport of HIV, and transmucosal transcytosis of HIV through tight epithelial lining. Importantly, colon epithelial cells, cervical uterine epithelial, and salivary gland epithelial cells have been reported to support HIV replication, and which may subsequently spread the virus. Secretory IgAs are the first line of defense against HIV. In this study, we used IgA monoclonal antibodies (MAb) against HIV gp160, to study the intra-epithelial neutralization, inhibition of HIV replication, and assembly/or budding using Vero C1008 cells expressing the polymeric immunoglobulin receptor (pIgR). Polarized Vero C1008 cells were transfected with HIV proviral DNA and intracellular neutralization mediated by MAbs of HIV was assessed. Samples from apical and basolateral supernatants, and cell lysate were assayed by infection of HeLa cells, that expressed human CD4 and the HIV long terminal repeat, and CEMx174 cells. Neutralization was measured by enumerating infected HeLa cells stained with X-gal and the HIV P24 level of infected CEMx174 cells. D47A and D19A IgA MAb, neutralize HIV infection conventionally, and potently inhibit intracellular HIV replication. While switch variant IgG, sharing the same V region, which will not be transported by pIgR, did not inhibit intracellular HIV replication though it can inhibit conventionally. No reduction was observed for $pIgR^{-}$ cells, incapable of endocytosing and transcytosing of IgA, that were treated with the same IgA MAbs. Confocal immunofluorescence microscopy showed prominent co-localization of HIV protein and D47A in agreement with intracellular neutralization. D10A, cannot neutralize HIV conventionally, and irrelevant IgA (anti-measles hemagglutinin) did not show co-localization or intracellular neutralization. In conclusion, during IgA transcytosis, HIV-specific IgA can interact and neutralize HIV replication intracellularly but not IgG.

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Changes in HIV Antiviral Phenotypic Drug Resistance During the HAART Era

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Objective: Patients newly diagnosed with HIV infection are treated initially with three-drug regimens, and many patients have been treated with drugs from three different classes of inhibitors. Optimization of treatment strategies has resulted in improved survival, but resistance develops readily in many patients, and correlates with availability of and exposure to antiretroviral therapy. We studied patient-level trends and variation in phenotypic resistance at several times during the HAART era in the four major US census regions. **Methods:** An automated percent sample of patient-level phenotypic resistance testing data from Virologic Inc. was analyzed for 7/01–6/03. Test results were assigned to zip codes, unique synthetic markers were generated for each record, and protected health information was deleted. (1) Data at the national level was analyzed comparing the average rate of phenotypic resistance seen for each antiviral class in 7/01–6/02 compared to 7/02–6/03, and (2) for each US census region compared to the US-wide average. Results were analyzed for statistically significant differences. **Results:** There were 500,000+ phenotypic drug resistance tests results in the combined study period. (1) No significant differences were seen in the average US-wide phenotypic resistance by class for 7/02–6/03 compared to 7/01–6/02. (2) Significant differences were seen for patient-level phenotypic resistance by class when comparing various Regions to the US-wide average, and when comparing regions to each other. **Conclusion:** While the average phenotypic resistance to HIV antivirals by class remained unchanged from the 12-month comparative periods, significant differences exist across regions. Clinicians need to consider regional variation in susceptibility to HIV antivirals in interpreting national findings and guidelines, and in patient-level prescribing decisions.

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Selection and Characterization of Virus Strains Resistant to a Novel Pyrimidinedione Nonnucleoside Inhibitor of HIV-1 and HIV-2

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SJ-3366 [1-(3-cyclopenten-1-yl)methyl-6-(3,5-dimethylbenzoyl)-5-ethyl-2,4-pyrimidinedione] is a highly active ($EC_{50} = 1$ nM, TI approximately 1 million) novel inhibitor of HIV-1 with the ability to inhibit both HIV entry and reverse transcription. In addition to its unique mechanism of action, SJ-3366 inhibits both HIV-1 and HIV-2, a property that distinguishes this specific NNRTI and related molecules from other NNRTIs. The dual mechanism of action and extended therapeutic range render SJ-3366 an attractive therapeutic agent for further clinical development. The effective treatment of HIV infection, however, is also dependent upon the potential of therapeutic agents to select for drug-resistant viruses. Therefore, it is critical to understand the resistance profile of any new anti-HIV agent prior to clinical use. The in vitro selection and phenotypic and genotypic characterization of resistant viruses has been extensively used as a means to predict the relative ease by which drug resistance-engendering mutations might appear in treated patients. Sensitivity and cross-resistance testing provide a means to rationally prioritize potential drug combinations that will yield effective anti-HIV therapeutic strategies. We have performed extensive resistance selection evaluations with the NNRTI SJ-3366 by serial passage of infectious virus in the presence of increasing concentrations of SJ-3366, resulting in viruses with complete resistance to the compound. At each passage, cell growth and viability, extent of virus productivity (intracellular and extracellular) and viral infectivity and cytopathic effect were quantified. Further characterization of the virus obtained at each passage included the evaluation of compound sensitivity, inhibition of virus attachment and reverse transcription, as well as sequencing of the viral reverse transcriptase and envelope. The resistant viruses obtained at each passage were also evaluated for their sensitivity to known attachment, fusion, reverse transcriptase and protease inhibitors. The results of these studies provide a highly extensive characterization of the resistance profile of SJ-3366, highlighting properties of the compound important for resistance, virus replication capacity and viral fitness and which will help determine its potential success as an anti-HIV therapeutic agent.

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Discovery and Characterization of Nonnucleoside Reverse Transcriptase Inhibitors Related to SJ-3366 with Efficacy Against Viruses Possessing the K103N Mutation in the Viral Reverse Transcriptase

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SJ-3366 is a nonnucleoside RT inhibitor distinguished by its extremely high therapeutic index (approximately 1 million), its activity against HIV-2 and its ability to inhibit both reverse transcription and viral entry. As an NNRTI, common resistance engendering mutations in the viral RT, especially the Y181C, Y188C, K101E and K103N amino acid changes, significantly reduced the efficacy of SJ-3366. Further, resistance to the second mechanism of HIV inhibition (virus attachment) was also reduced during the selection of resistant virus. A series of analogs of SJ-3366 was screened for antiviral properties that would reduce the rate of resistance selection and potentially yield a more potent and robust compound for clinical development. Our evaluations of the antiviral efficacy of the SJ series yielded 15 compounds with high therapeutic indices (greater than 500,000) and three of these inhibitors were active against viruses possessing the K103N amino acid change in the RT. In light of the significant biological effect of this particular mutation on nearly all NNRTIs, further evaluation of these SJ compounds was performed. The K103N active molecules maintain high therapeutic efficacy against HIV-1, are active against HIV-2, and are similarly inhibitory to reverse transcription and virus entry. Characterization of the sensitivity of these compounds to viruses with other NNRTI resistance-engendering mutations and evaluation in resistance selection and combination therapy assays provides further evidence of the therapeutic potential of these compounds.

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Sensitive Methodology to Assess Resistance, Fitness, Replication Capacity and Compensatory Mutations of Reverse Transcriptase Inhibitor Resistant Viruses

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The rapid selection of drug-resistant virus strains in HIV-infected patients during antiviral therapy is a primary reason for treatment failure with both nucleoside and nonnucleoside RT inhibitors. Although resistance would appear to be a detrimental outcome of antiviral therapy, it may be possible to select for resistant viruses with reduced replication capacity and/or fitness. Resistance-engendering mutations may yield beneficial therapeutic effects if the se-

lected mutations cause reductions in the rate and/or extent of virus replication. Reduced virus replication may effectively prolong the interval between initial HIV infection and AIDS, allow the immune system to more effectively deal with the virus, and allow more effective therapeutic intervention with other HIV inhibitory agents. Our laboratory has developed assays which allow us to evaluate the relative rate of resistance selection between antiviral compounds, and to compare wild-type and resistant virus strains with regard to their replication kinetics and capacity and their relative fitness, as well as identifying the potential contribution of compensatory changes in the virus which result in enhanced replication of low fitness viruses. These assays employ both laboratory-derived and clinical strains of HIV or NL4-3 virus with specific amino acid changes introduced into the reverse transcriptase by site directed mutagenesis. Using competitive viral replication assays, we have shown that virus containing point mutations that confer resistance to nucleoside and nonnucleoside RT inhibitors may exhibit reduced or increased rates and extents of virus replication or may exhibit no change in replication. The relative growth potential of the mutant virus was also compared to wild-type virus in the absence and presence of drug selective pressure. These results demonstrated that drug-induced mutations in the reverse transcriptase have both positive and negative effects on the ability of HIV to replicate in human cells. Further evaluation of each mutation, alone or in combination, may prove valuable in designing therapeutic strategies for HIV-infected patients.

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Novel Tyrosine Derived HIV Protease Inhibitors Possessing Potent Anti-HIV Activity and Remarkable Inverse Antiviral Resistance Profiles

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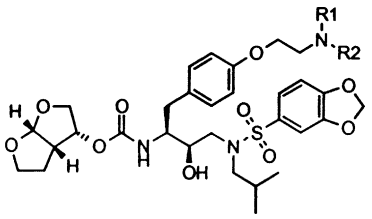

Through their use as an integral component of combination anti-retroviral therapy, HIV protease inhibitors (PIs) have transformed HIV into a much more manageable disease than it was prior to their introduction in the mid-1990s. However, due to the emergence of viral strains resistant to the current generation of PIs, the discovery of newer agents capable of treating PI-resistant HIV is becoming increasingly important. Our efforts toward this end led to the identification of a novel series of arylsulfonamides possessing low nanomolar IC_{50}'s against wild-type HIV virus. In addition, a number of these compounds showed a remarkable inverse antiviral resistance profile when tested against two multi-PI resistant viral strains. Compounds in this structural class showed *increases* in activity of up to fourfold when tested against the multi-PI resistant virus D545701 as compared to wild-type virus. This compares to ninefold and greater than threefold *reductions* in activity for the currently used PIs indinavir and nelfinavir, respectively. A detailed discussion of the synthesis and antiviral structure–activity relationships of these compounds will be presented in this poster.

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HIV-1 Resistance to ADS-J1

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ADS-J1 is a low molecular weight compound selected for its (ability to interfere with the association of the N- and C-terminal heptad repeat regions of HIV-1 gp41 envelope glycoprotein. Since ADS-J1 is a polysulfonic acid compound, it is interesting to know whether it, like other polyanionic anti-HIV compounds blocks the binding of HIV-1 to CD4 cells, to test the anti-HIV activity of ADS-J1 against HIV strains that have been made resistant to polyanionic HIV binding inhibitors and study the development of resistance as a means to understand its mechanism of action. ADS-J1 was active against T-20- and C-34-resistant HIV-1 isolates with similar potency to the wild-type H1V-1 NL4-3 strain (EC_{50} 0.6, 0.3 and 0.4 μg/ml, respectively). ADS-J1 (10 μg/ml) could not block the binding of an HIV strain that was made resistant to AR177, a negatively charged oligonucleotide that blocks HIV binding, and is cross-resistant (>100-fold) to dextran sulfate (DS), a known polysulfonic HIV binding inhibitor. However, ADS-J1 blocked AR177-resistant virus fusion and replication (EC_{50} 1.5 μg/ml), suggesting that ADS-J1 has mechanism of action different from the polyanionic HIV binding inhibitor AR177. Selection of ADS-J1-resistant HIV from an AR177-resistant virus showed the emergence of several mutations in the V3 region of the env gene, suggesting that selective pressure was still necessary to overcome the effect of ADS-J1 on gp120. Notably, ADS-J1 induced a change in net charge in the V3 loop from +9 to +5. A mutation (L335) in the gp41 coding sequence, previously associated to HIV-resistance to the gp41 inhibitors T-20 and C-34, emerged as consequence of ADS-J1 selective pressure. Our results suggest that negatively charged compounds, such as

ADS-J1, could interact with gp41 but gp120 is the principal target of their anti-HIV activity. A reduction of net charge in the V3 loop could be associated to a switch of HIV coreceptor use.

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Variability in the Use of HIV Phenotypic Resistance Testing by Specialty of Practitioner

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Background: HIV treatment has involved infectious disease specialists and other specialties. We studied the relative use of phenotypic resistance testing among specialties and by different regions in the US over time. **Methods:** Phenotypic resistance testing data from Virologic was aggregated for 7/01–6/03. Tests were assigned to each ordering physicians. Self-reported specialty was identified and an automated sample generated. Specialties were grouped as infectious disease, internal medicine, primary care (family practice and general practice) and all others. 'Unspecified' physician records were deleted. Data were analyzed for statistically significant differences in: (1) the average use of resistance testing by each specialty in 7/01–6/02 compared to 7/02–6/03 across the US and (2) the use of resistance testing in the each US census region compared to the average US-wide use for each respective specialty. **Results:** There were approximately 2600 physicians in the sample for which 250,000 drug resistance tests were conducted. (1) Across the US the average use of phenotypic resistance testing by Infectious Disease statistically increased 5% ($P \leq 0.05$) for 7/02–6/03 compared to 7/01–6/02 and the use by other specialties decreased. (2) Within each specialty there were statistically significant differences by geographic region in the use of phenotypic resistance testing. **Conclusions:** Based on its relative ease of interpretation compared to genotypic information we might have predicted that the use of phenotypic resistance testing would increase for non-Infectious Disease specialists. The reverse was true, i.e. it increased for Infectious Disease relative to other specialties use. Significant differences in the use of phenotypic resistance testing were also seen by regions. Contributing factors may include increasing adoption of phenotypic resistance testing by Infectious Disease specialists, variation in patient severity and financial constraints.

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Mechanism of Anti-HIV Activity of Dioxolane Nucleosides Against 3TC-Resistant HIV-1 Reverse Transcriptase-Molecular Modeling Approach

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The majority of AIDS patients are currently taking lamivudine as part of combination therapy. Therefore, development of the drug-resistant mutant against the drug is a serious problem for the management of HIV infection. Since the discovery of β-D-dioxolane-2,6-diaminopurine (DAPD or Amdoxovir) is active against AZT- as well as 3TC-resistant mutants, several other nucleosides with a dioxolane moiety have been synthesized. Among the series of dioxolane nucleosides, the thymidine, and 5-fluorocytidine analogs showed potent anti-HIV activity against 3TC-resistant mutant RT. Thus, it is of great interest to understand the role of dioxolane moiety in the anti-HIV activity against 3TC-resistant mutant. Various D-dioxolane nucleoside triphosphates and 3TC triphosphate were docked into the active site of WT as well as 3TC-resistant RT, and the resulting complexes were energy-minimized. The M184V mutation imposes steric hindrances to the incoming nucleoside triphosphates as well as the nearby primer chain. Therefore, if the nucleoside triphosphate–RT complex cannot provide enough conformational flexibility to escape from the steric hindrance, it results in an abortive binding state. Our molecular modeling studies indicated that D-dioxolane nucleoside triphosphate–RT complexes, unlike 3TC triphosphate–RT complex, not sterically hindered the bulky side chain of Val184, and maintain the favorable binding modes through the interaction of 3′-oxygen with active site residues, such as Arg72 or Tyr115. This information may be useful for the discovery of additional nucleosides with improved activity against 184V mutants.

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Virus Resistance to the CXCR4 inhibitor AMD070 Develops Slowly and Does Not Induce a Co-receptor Switch

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AMD070 is a novel orally bioavailable CXCR4 antagonist that potently inhibits X4 viruses at a 50% effective concentration (EC_{50}) of 1–20 nM in T cell lines, CXCR4-transfected cell lines and PBMCs. As occurrence of viral resistance is a major concern in the development of anti-HIV drugs, resistance development against AMD070 and co-receptor use was therefore examined in vitro. MT-4 cells were infected with HIV-1 NL4.3 in medium containing the drug at different concentrations. Cultures were incubated at 37 °C until an extensive cytopathic effect was observed (∼5 days). The culture supernatant was used for further passage of virus in MT-4 cells in the presence of

increasing concentrations of drug. PBMCs (obtained from healthy donors) were infected with a pure HIV-1 X4 clinical isolate in medium containing the drug at different doses. Cell cultures were incubated until a cytopathic effect (syncytium formation) was observed (~10 days), p24 viral Ag production was determined and PBMCs were re-infected in the presence (and absence) of compound. Co-receptor use was determined in transfected cells expressing CD4 and CXCR4 or CCR5. After 60 passages of NL4.3 virus in MT-4 cells, virus was isolated which was inhibited by AMD070 at an EC_{50} of 195 nM, whereas the WT virus passaged in MT-4 cells without the drug was inhibited at an EC_{50} of 9 nM (>20-fold resistance). After 16 passages of the clinical X4 isolate in PBMCs an EC_{50} of 135 nM was obtained, whereas with the virus passaged in the cells without the drug an EC_{50} of 15 nM was obtained (less than 10-fold). In a similar manner, as the original virus, the viruses isolated in the presence of the drug, were able to infect transfected cells expressing CXCR4 but not cells expressing CCR5. Thus, the X4 NL4.3 strain (300 days cultured in the presence of AMD070) and the X4 isolate (160 days in the presence of drug) proved capable of overcoming slowly and only partially the inhibitory effect of the CXCR4 inhibitor, but still needed CXCR4 to enter and infect the cells. HIV-1 escape from the use of a CXCR4 inhibitor does not induce a co-receptor switch.

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Anabolism of Amdoxovir: Phosphorylation of Dioxolane Guanosine and Its 5′-Phosphates by Mammalian Phosphotransferases

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Amdoxovir [(−)-β-D-2,6-diaminopurine, DAPD], the prodrug of dioxolane guanosine (DXG), is currently in Phase I/II clinical development for the treatment of HIV-1 infection. In this study, we examined the phosphorylation pathway of DXG using 15 purified enzymes from human (eight), animal (six), and yeast (one) sources, including deoxyguanosine kinase (dGK), deoxycytidine kinase (dCK), high K_m 5′-nucleotidase (5′-NT), guanylate kinase (GMPK), nucleoside monophosphate kinase (NMPK), adenylate kinase (AMPK), nucleoside diphosphate kinase (NDPK), 3′-phosphoglycerate kinase (PGK), creatine kinase (CK) and pyruvate kinase (PK). In addition, the metabolism of ^{14}C-labeled DXG was studied in CEM cells. DXG was not phosphorylated by human dCK, and was a poor substrate for human dGK with a high K_m (7 mM). Human 5′-NT phosphorylated DXG with relatively high efficiency (4.2% of dGuo). DXG-MP was a good substrate for porcine brain GMPK with a substrate specificity that was 10% of dGMP. DXG-DP was phosphorylated by all of the NDPK, PGK, CK and PK tested. The BB-isoform of human CK showed the highest relative substrate specificity (47% of dGDP) for DXG-DP. In CEM cells incubated with 5 μM DXG for 24 h, 0.015 pmol/10^6 cells of DXG-TP was detected as the primary metabolite. Our study demonstrated that 5′-nucleotidase, guanylate kinase, creatine kinase, and nucleoside diphosphate kinase could be responsible for the activation of DXG in vivo.

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Evaluation of Virologic Response and Resistance Profile in Treatment Experienced Patients Receiving Amdoxovir

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Background: The presence of mutations to nucleoside reverse transcriptase inhibitors (NRTI) in antiretroviral treatment (ART) experienced HIV-1 infected patients may compromise viral response to subsequent regimens. Amdoxovir (DAPD) is a nucleoside analogue under development for the treatment of HIV which has demonstrated activity in vitro against HIV containing NRTI associated mutations. **Methods:** DAPD-150 is a phase I, open-label study of 18 HIV infected, ART experienced patients that were randomized to receive DAPD (300 or 500 mg b.i.d.) either in combination with their current regimen or as part of an optimized regimen of ART. Virologic failure (VF) was defined as a lack of virological response, failure to reach a 0.5 $\log_{10}$ decrease in viral load (VL) from baseline (BL) by week 8, or loss of response (rebound to within 0.5 $\log_{10}$ in plasma HIV RNA from BL). Genotypic data were obtained on BL HIV-1 RNA for 17 of 18 patients, and at time of VF. **Results:** Of the 18 patients enrolled in the study, 9 had DAPD added on to their current regimen, and 9 received DAPD as part of an optimized regimen. Three patients in the add-on cohort and one in the optimized cohort discontinued prior to week 8 and were censored from analysis. BL genotypic data obtained on the remaining 14 patients showed a median of three NRTI mutations per group. At week 8, median log change in HIV VL from baseline in the add-on cohort was −0.63 (0.42 to −1.82, $n = 6$), as compared to −2.10 (0.62 to −2.5, $n = 8$) for patients in the optimized cohort. Two patients from each cohort failed to achieve >0.5 $\log_{10}$ drop in HIV RNA by week 8. Genotypic analysis demonstrated the addition of L741 for one patient in the add-on group and no mutational changes for the other three patients. For the remaining 10 patients who continued therapy past 8 weeks, 3 went on to experience protocol defined VF. Genotypic analysis demonstrated the addition of mutations at K101E,

G190A, and T215Y for one patient in the add-on cohort and no changes for the other patient, while the one patient experiencing VF in the optimized cohort added mutations at L741 and M184V. **Conclusions:** When DAPD is used as a new agent or part of an optimized ART, a marked reduction in HIV VL in ART experienced patients with NRTI associated mutations is observed.

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MIV-150, A Potent HIV-1 Inhibitor Suitable for Use in Microbicides

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MIV-150 is a non-nucleoside reverse transcriptase inhibitor (NNRTI) with a tight binding to HIV reverse transcriptase. This results in an inactivation of free virus particles as well as an inhibition of HIV replication at low nanomolar concentrations. MIV-150 is a more potent inhibitor than sustiva of NNRTI resistant mutants also in the presence of human serum. The rate of in vitro resistance development to MIV-150 is three times slower than for sustiva. MIV-150 has shown a potent activity against SHIV in monkeys, prevented infection when dosed after SHIV inoculation and showed a good profile in preclinical safety and toxicology studies. A phase I clinical study showed a low oral bioavailability. Taken together, these results strongly indicate the use of MIV-150 in a topical microbicide to prevent HIV transmission and combination studies with Carraguard has shown synergy in vitro.

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Design and Preclinical Development of Dendrimer Based Topical Microbicides for HIV and STI Prevention

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Since 1996, Starpharma has been committed to the discovery and development of pharmaceuticals based on dendrimer nanotechnology. Dendrimers are a new class of macromolecule characterized by highly branched, three-dimensional architectures that are assembled in a precise, step-wise manner.[1] This controlled synthesis allows the assembly of structures that radiate out in generations from a central initiator core which results in a single macromolecular entity. This is in contrast to the polydisperse nature of alternative macromolecular structures, such as traditional polymers.

In developing dendrimers as pharmaceuticals, Starpharma has had a focus on the prevention of HIV and sexually transmitted infections (STIs) through the development of microbicide candidates. Early examples of Starpharma's dendrimers had activity against HIV[2] and herpes simplex virus-2 (HSV-2)[3] which triggered a lead candidate optimization process. This work resulted in a series of optimized dendrimer based microbicides being identified[4] from which SPL7013 emerged as a development candidate. Following a range of preclinical studies, Starpharma submitted an Investigational New Drug application (IND) for SPL7013 gel (VivaGel^{TM}) to the United States Food and Drug Administration (FDA). VivaGel represented the first time a dendrimer based pharmaceutical had been submitted to the FDA. Phase I clinical trials are currently in progress.

This presentation will discuss this dendrimer based microbicide discovery and development process.

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In Vitro Strategies for the Inhibition of Sexually Transmitted Diseases: Preclinical Development of Topical Mono- and Combination Microbicide Therapies

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One of the current challenges in anti-infective drug discovery is the inhibition of the sexual transmission of infectious organisms between sexual partners. We have developed a series of microtiter-based, high-throughput assays to evaluate the ability of anti-infective compounds to be used as topical microbicides alone or in combination with agents active against the same or different infectious organisms. These assays include cell-based and biochemical assays that quantify the inhibition of replication of the infectious organism. These assays have served to define a variety of classes of active inhibitors, including polyanionic molecules, surfactants, natural products, peptides, proteins, heterocycles, virucidal agents, and other anti-infective agents. Efficacy and toxicity (especially against common flora of the vagina, such as *Lactobacillus*) of candidate compounds is determined in conditions that mimic the type of environment in which the compounds will be required to work, including the effects of pH and mucopolysaccharides, as well as other conditions involving time of infection, treatment schedule and multiplicity of infection. An integral part of the assessment of any topical microbicide candidate is demonstration of appropriate range and mechanism of action compatible

with a topical microbicide. Range of action assays evaluate the ability of candidate compounds to act against a variety of wild-type, drug-resistant, laboratory-derived, and clinical strains of virus. Mechanism of action assays, encompassing both biochemical/enzymatic and cell-based assays, are employed to further define the activity of the compound in intact cells. Assays are performed to determine the relative ease of selecting for drug resistant virus strains in culture and to define the interactions of the compounds when used in combination with other active agents. Finally, candidates can be assessed in animal models for in vivo efficacy. Representative results obtained with a variety of compounds will be presented.

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Topical Microbicide Therapies Involving the Use of Non-nucleoside Reverse Transcriptase Inhibitors: Combination Therapy and Resistance

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The development of safe and effective topical microbicides is an urgent medical need throughout the world in order to inhibit the sexual spread of HIV and other sexually transmitted diseases. Although it has been presumed that topical therapies would allow higher concentrations of any anti-infective compounds to be used, thereby limiting the emergence of drug-resistant virus strains, it is likely that topical therapies will be required to prevent the transmission of drug-resistant virus strains which are increasing in frequency in the population. In addition, topical microbicides may indeed act to select for rare resistant strains from within virus swarms upon transmission, effectively acting as a barrier to wild-type virus while permitting transmission of resistant organisms. We have investigated the potential use of combination therapeutic strategies involving topical microbicides with regard to their ability to inhibit wild-type and drug-resistant virus strains. Specifically, we have performed in vitro assays to evaluate the potential for therapeutic use of nonnucleoside reverse transcriptase inhibitors (thiocarboxanilide, calanolide A and SJ-3366) in CD4-dependent and CD4-independent virus transmission assays. In general, the NNRTIs interact in an additive to synergistic manner with all compounds tested, with no evidence of synergistic toxicity or antagonism. Interestingly, each of the tested NNRTIs exhibits a distinct profile of combination efficacy when tested with wild-type and/or drug-resistant virus strains. In nearly all cases synergistic inhibition of both wild-type and drug-resistant virus strains was observed, although higher concentrations of the compounds were required to achieve inhibition of the resistant strains. The results of our combination microbicidal and resistance selection assays will be presented.

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Novel Acyclic Nucleoside Phosphonate Analogues as Potent and Selective Anti-HBV Agents

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Various novel acyclic nucleoside phosphonate analogues, in which the base consists of a pyrimidine preferably containing an amino group at C-2, an amino or hydroxy group at C-4 and a [2-(phosphono-methoxy)ethyl]oxy (PMEO), [(*R*)- or (*S*)-2-(phosphonomethoxy)-propyl]oxy (PMPO) or (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl]-oxy-[(*S*)-HPMPO] group at the C-6 position, were evaluated for their potential effect on the replication of HBV using the HBV-inducible cell lines HepAD38 (wild-type) and HepAD79 (3TC-resistant). The antiviral effect was quantified by measuring viral DNA in culture supernatant at day 6 post-induction by real-time quantitative PCR. The 6-PMEO-2,4-diaminopyrimidine (compound **1**) and its derivatives (compound **2**, (*S*)-HPMPO-2,4-diaminopyrimidine; compound **3**, 6-PMEO-2,4-diamino-5-methylpyrimidine; compound **4**, 6-PMEO-2-amino-4-hydroxypyrimidine) showed potent antiviral activity against both wild-type and 3TC-resistant viruses. Their anti-HBV activity was comparable to that of the reference compounds, PMEA and PMPA. Replacing the ether group at C-6 by a thioether function (compound **5**), resulted in a compound which still exhibited potent anti-HBV activity. The 2,4-diaminopyrimidine moiety appears to be a prerequisite for the anti-HBV efficacy. The presence of other substituents (H, HO or CH_3) at the C-2 position (compound **6**, 6-PMEO-4-aminopyrimidine), largely reduced the antiviral activity. When the 2-(phosphonomethoxy)ethyl or 2-(phosphonomethoxy)propyl side-chain was attached to the pyrimidine N-1 (the natural position of nucleos(t)ides), the anti-HBV activity was completely lost. The PMEO, PMPO and HPMPO pyrimidine analogues had little or no cytotoxic or cytostatic activity. Several compounds in this series thus appear to exhibit potent and selective activity against both wild-type HBV and the lamivudine-resistant YMDD variant of HBV.

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Investigation into the Ability of GBV-B to Replicate in Various Immortalized Cell Lines

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GB virus B (GBV-B) is the closest related virus phylogenetically with the hepatitis C virus (HCV) and is an attrac-

tive surrogate model system for HCV drug development efforts. Unfortunately, the model system has not been widely utilized since the virus can only be grown in the primary hepatocytes of certain non-human primates (NHP). We grew GBV-B in NHPs and then used this virus to try to infect a variety of immortalized cell lines. We also used lipofection reagents to transfect GBV-B into cells in case the virus could replicate in, but not enter the cells due to a lack of receptors. TaqMan RT–PCR was used to assess replication. The following cell lines were negative for GBV-B production in these expts: HepG2, Hep2-287, Huh7, E13, B95-8, Vero, MDBK, H5, H8, H16, LX2, human PBMCs, and cynmolgous monkey and human primary hepatocytes. Experiments have been undertaken to immortalize NHP primary hepatocytes using SV40 large T-antigen and cell fusion. Infectious full-length GBV-B RNA could be transfected into Vero, Huh7 and HepG2 at high efficiency, however, there was no evidence for replication. We are currently working on the construction of GBV-B stable cell lines.

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A Comparative Analysis of Two Hepatitis B Virus (HBV) Viral Load Assays: Real-Time PCR versus Hybrid Capture

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Introduction: The advent of new, potent nucleoside analogs for the treatment of HBV infection has increased the need for more sensitive HBV viral load (VL) assays that are reliable and can accurately detect HBV VL over a large dynamic range. A comparative analysis of a commercially available HBV VL assay, Digene® HBV Hybrid Capture II Test (Digene), and an in house developed Real-Time PCR assay using TaqMan Technology was performed to evaluate the concordance, correlation and clinical utility of both assays. **Methods:** HBV DNA was measured using the Digene® assay, a signal amplification hybridization assay using chemiluminescent detection and quantification of HBV DNA in serum, which has a six log dynamic range and limit of detection (LOD) of 4700 copies/ml (c/ml). The Real-Time PCR based assay is an absolute quantification of nucleic acids using a standard curve and has a seven log dynamic range and LOD of 200 copies/ml. Serum samples from 13 patients enrolled in phase I/II clinical studies of an experimental nucleoside analog were quantified on each of the two assays, for a total of 128 time points. Statistical analysis of the VL data was performed on a $1\log_{10}$ scale to evaluate the linear correlation and the concordance over the dynamic range of both assays. **Results:** The Digene and Real-Time assays correlated closely in the overlapping range of 4700 to 1.7×10^9 c/ml, ($R = 0.975$). Concordance in this range was also strong, with 83% of the Real-Time PCR values within 10% of the corresponding Digene value. As expected, poor concordance (21%) was seen in the range of 200–4700 c/ml ($R = 0.422$) due to the increased sensitivity of the Real-Time assay. Of the 23 samples with HBV DNA values undetectable by Digene, 20 (87%) were detectable using Real-Time PCR. **Conclusion:** The hybrid capture and in-house developed Real-Time PCR assays were highly concordant and closely correlated over the dynamic range of the Digene assay (1.7×10^9 to 4700 c/ml). The increased sensitivity of the Real-Time PCR assay allows assessment of patient's response to therapy over a wider range of viral loads.

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Highly Sensitive and Accurate Real-time PCR Assay for Hepatitis B Viral Load Quantification

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Background: Hepatitis B virus (HBV) DNA viral load is an important indicator for evaluating drug efficacy. With the advent of new potent drugs and combination therapies, it has become important to reliably monitor very low levels (less than 10^3 copies/ml) of HBV DNA in serum. **Method:** HBV DNA was extracted from 200 ul of clinical serum or laboratory samples and the number of HBV copies present was determined with a real-time PCR assay using TaqMan technology. Primers and a probe detecting a conserved region in the HBV X gene were employed. Studies were performed evaluating accuracy, precision, interfering substances, sensitivity and reportable range using samples representing HBV genotypes A through G. **Results:** The validated real-time PCR assay can reliably quantify 3×10^2 to 1×10^8 copies/ml of HBV in genotypes A through E in human serum samples. It demonstrated a bias (difference from target value) range of 1–36% across all samples tested, with an increased bias at lower viral loads. The less prevalent HBV genotypes F and G cannot be accurately quantified in this assay. For genotypes A–E, the assay demonstrated an excellent accuracy with 95%(137/144) of observed values within the acceptance criteria (target value $\pm 50\%$ for samples $> 10^3$ copies/ml and $\pm 60\%$ for samples $< 10^3$ copies/ml). A correlation of observed to expected values of $R^2 \geq 0.99$ was achieved. The assay also showed an exceptional reproducibility across the reportable range with an intra-assay precision range of 0.4–18.7%CV for samples $> 10^3$ copies/ml and 6.5–40.5%CV for samples $< 10^3$ copies/ml and an inter-assay precision range of 3.9–35.0%CV for samples $> 10^3$ copies/ml and 16.5–27.2%CV $< 10^3$ copies/ml. No interference was observed with the agents tested. Testing for reagent and sample stability is in progress. **Conclusions:** We have developed and validated a real-time PCR assay that quantifies HBV viral loads over 8 orders of magnitude with a lower limit of detection of 200 copies/ml in human serum.

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Molecular Modelling of Hepatitis B Virus Polymerase: Comparison of Adefovir, Entecavir and Lamivudine Resistance

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The antiviral treatment of chronic hepatitis B is limited by the selection of antiviral resistance mutations. To gain an understanding of the hepatitis B virus (HBV) polymerase and also mutations associated with resistance, a three-dimensional model of the HBV reverse transcriptase core region based on homology with HIV was created. Primary resistance to lamivudine occurs at rtM204I/V (C domain) ±rtL180M (B Domain) of the polymerase. Previous molecular modeling studies have proposed that these mutations cause steric hindrance between the changed amino acid (rtM204I/V) and the sulphur atom in the oxathiolane ring of LMV-tri phosphate (TP) [Das et al. 2001 JV 75:4771]. Recently, we have identified resistance to both adefovir (ADV) and entecavir (ETV). Resistance to ADV is associated with a mutation in the D Domain at rtN236T, that may potentially result in the perturbation of the ADV-TP binding site. While the resistance profile to ETV is complex and is in association with the LMV resistance mutations. Two patients have been identified with ETV and LMV resistance. The first patient had the rtL180M, rtM204V and rtVl73V/L were present at study entry, with substitutions rtI169T (B Domain) and rtM250V (E Domain) selected on ETV treatment. The second patient had polymerase changes rtS78S/T, rtL180M, rtV173V/L, rtT184T/S and rtM204V at study entry and selected rtA38E, rtT184G (B Domain) and rtS202I (C domain) during ETV treatment. Molecular modeling is an important tool in understanding primary antiviral resistance to these antiviral agents. It may provide insights for the improved treatment of patients on monotherapy and multiple combinations of antiviral agents.

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Ribavirin and Mycophenolic Acid Potentiate the Activity of Entecavir against Hepatitis B Virus

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Entecavir (ETV), a cyclopentyl 2′-deoxyguanosine nucleoside analogue, is a potent and selective inhibitor of hepatitis B virus (HBV) replication. Here we report that mycophenolic acid [the active metabolite of the immunosuppressive agent mycophenolate mofetil (MMF)] and ribavirin markedly potentiate the anti-HBV activity of ETV. Ribavirin and mycophenolic acid are both inhibitors of inosine 5′-monophosphate dehydrogenase and cause a depletion of intracellular dGTP levels. ETV triphosphate (ETV-TP), which competes with dGTP for inhibition of the hepadnaviral DNA polymerase, effectively suppresses the priming and elongation steps during HBV replication. It may be assumed that ETV-TP inhibits these steps more efficiently in the presence of reduced levels of intracellular dGTP. Our observations may have implications for those (liver) transplant recipients that receive MMF as (part of their) immunosuppressive regimen and that, because of de novo or persistent infection with HBV, need specific anti-HBV therapy such as ETV. Also ribavirin, which is being used extensively for the treatment of HCV infections, may be able to increase the potency of ETV against HBV infections in the clinical setting.

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Long-term Adefovir Dipivoxil (ADV) Demonstrates Sustained Efficacy in Chronic Hepatitis B (CHB)

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ADV has previously been shown to demonstrate efficacy and safety in treatment of HBeAg positive, HBeAg negative and lamivudine-resistant (LAM-R) CHB for treatment periods of 48 and 96 weeks. Resistance emergence was shown to be delayed and infrequent. **Objective:** To evaluate the long-term safety and efficacy of ADV over 144 Weeks. **Methods:** Long-term efficacy and safety data over 144 weeks were obtained in HBeAg negative CHB patients and HBV/HIV co-infected patients with LAM-R. HBV DNA (Roche Amplicor PCR) and ALT levels were analyzed. In the HIV/HBV co-infection study ADV was added to ongoing LAM. **Results:** In the HBeAg negative study at entry median HBV DNA was 7.08 $\log_{10}$ copies/ml and median ALT was 99 IU/l (2.3 × ULN). In the HBV/HIV co-infected study median HBV DNA was 8.75 $\log_{10}$ copies/ml and the median ALT was 81 IU/l (2.2 × ULN).

	HBeAg−		HBV/HIV	
	W48 (n = 79)	W144 (n = 67)	W48 (n = 31)	W144 (n = 2)
Median change: HBV DNA (log_{10} copies/ml)	−3.54 (n = 78)	−3.63	−4.0	−5.5
% HBV DNA undetectable by PCR (<1000 copies/ml)	68 (n = 78)	79	6	46
Median change: ALT (IU/l)	−60	54	−16	−46
% ALT normalized[a]	75	69	19	64

[a] For patients with ALT > ULN at baseline.

No resistance was seen at 48 weeks in either study. Resistance emergence over longer durations has been infrequent (2% at 96 weeks, 3.9% at 144 weeks in an integrated analysis across four studies). ADV was well-tolerated. Three patients in the HBeAg negative study and two patients in the HIV/HBV co-infected study had confirmed elevations in serum creatinine $\geq$ 0.5 mg/dl over 144 weeks. All resolved, two on treatment and three following study drug interruption or discontinuation. **Conclusions:** Treatment with ADV over 144 weeks resulted in sustained reductions in serum HBV DNA levels with an increasing proportion of patients achieving HBV DNA undetectability. ALT change and normalization was sustained. Resistance emergence up to 144 weeks remained infrequent. The safety profile of ADV during three years of treatment was similar to that seen in the first two years.

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Characterisation and Functional Analysis of Hepatitis B Virus Mutants Selected in Patients with Severe Hepatitis During Long Term Lamivudine Therapy

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The ongoing use of antiviral therapy in the presence of viral replication results in continuous selection of potentially compensatory mutations within the hepatitis B virus (HBV) genome. The aim of this study was to characterize the HBV genome from five patients with virological breakthrough and disease exacerbation during lamivudine (LMV) treatment. The entire HBV polymerase from 44 serial specimens was amplified and sequenced. In addition to the well characterized mutations at rtM204I/V (C domain) ±rtL180M (B Domain), an rtL80I (A Domain) mutation was the main change detected in these patients. Molecular modelling of the HBV polymerase revealed that the rtL80 maps to the nucleotide binding pocket of the polymerase, but does not directly interact with LMV via steric hindrance. It may affect viral replication by altering the processivity of the enzyme. The relative replication yield phenotype and antiviral sensitivity to LMV was compared for HBV clones encoding the LMV resistant mutation in combination with the rtL80I. The rtL80I mutation acted as a compensatory mutation and significantly increased the replication yield phenotype of the rtM204I clone. The combination of the rtM204I/V mutations with the rtL80I resulted in a reduced antiviral sensitive phenotype to LMV. As selection of rtL80I was found to have a higher replication yield phenotype in vitro, it may have contributed to the severe hepatitis observed in these patients during long-term LMV treatment. These observations argue strongly against the practice of using antiviral therapy in patients viraemic with resistant HBV due to the ongoing selection of compensatory mutations.

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The Virological Basis of the Hypersensitivity of HBV, HCV and Pestiviruses Sensitivity to Glucosidase Inhibitors

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Secretion of hepatitis B, C and BVDV from infected cells is prevented by glucosidase inhibitors at concentrations that have little impact upon most cellular functions. Moreover, secretion of HBV from tissue cultures which glucosidase has been inhibited with the recently US FDA approved glucosidase inhibitor, NB-DNJ (now referred to as Zavesca), fail to secrete virus for several days after the inhibitor has been removed and glucosidase function has returned to baseline levels. While this has implications regarding the durability of an antiviral response to this class of compounds, it also suggested mechanisms of antiviral action that will be discussed. Since the M envelope polypeptide of HBV is folded via a calnexin/glucosidase mediated pathway, it has been assumed that the sensitivity of HBV to glucosidase inhibitors was mediated by the HBV M. protein. By pulse chase, western, genetic and immunostaining, this study shows that the biogenesis and degradation of HBV L and M is aberrant, relative to cellular glycoproteins, in cells incubated with glucosidase inhibitor and thus both play a role in the antiviral mechanism of this class of compounds.

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Glycolipid Mimetics Induce an Innate Host Response and Have Anti-HBV and HCV Activity, In Vitro

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A new chemical family of glycolipid mimetic type molecules, characterized by an imino sugar head group and alkylated side chains of between 5 and 10 carbon lengths, is shown here to have sub micro molar activity against HBV, HCV replicons and BVDV, in vitro with selectivity indexes exceeding 100. Although these molecules, called alkovirs, prevent viral replication, they do not apparently inhibit viral enzymes. Rather, they appear to inhibit viral assembly or morphogenesis in a manner analogous to interferons. Consistent with that observation, we also show that they induce members of the 2′,5′ OAS family. One member of the alkovirs family is in Phase II clinical trials for treatment of hepatitis C. Another has been tested for its ability to inhibit woodchuck hepatitis virus, in vitro and in vivo. The results of that study and the implications for the development and understanding of the alkovirs, will be discussed.

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A Cell-based Assay for Screening of Potential Antiviral Agents Against HCV

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In vitro cultures of cell-lines capable of supporting HCV propagation are critical for understanding viral replication and pathogenesis, as well as for evaluating anti-HCV compounds. In trying to mimic as closely as possible the viral life cycle, we took the approach of infecting human cell-lines with HCV derived from HCV patients. The requirements from this system were to clearly demonstrate viral expression, viral replication, capability of emergent particles to infect new cultures and ability to use the system for testing potential therapeutics. **Methods:** We used two human hepatoma cell-lines Huh7 and FLC-4. FLC-4 cells were stably transfected with EBV-EBNA I to enhance HCV replication and expression. Cells were infected by incubation with HCV-positive serum and sub-cultured weekly. Viral infection was followed by detection of HCV-RNA. HCV infection of cells was further confirmed by in situ hybridization. Viral replication was assayed by detection of (−) strand RNA in infected cells. **Results:** HCV-RNA could be detected in cells up to four weeks post infection. HCV-RNA was detected in culture supernatants of infected cultures as well, suggesting emergence of viral particles. The existence of HCV infectious particles in cell cultures is supported by data showing ability to re-infect naive cells. Presence of (−) strand RNA was observed in cells but not in cultured supernatant. Feasibility of using this cell-based system for evaluating efficacy of antiviral agents has been tested using small molecules as well as human monoclonal antibodies directed against the envelope proteins of HCV. Treatment of infected cells with IFN-alpha resulted in significant reduction of HCV-RNA levels in cells. In addition, human monoclonal antibody against E2 inhibited HCV infection when pre-incubated with the infectious serum. The assay has been successfully used in parallel with our Trimera™ model to test new potential antiviral agents against HCV.

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Development of a Sensitive and Rapid Real-Time RT-PCR Assay Using Lightcycler for Quantitative Analysis of Hepatitis C Virus RNA

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In order to develop a sensitive and quantitative RT–PCR assay for detection of HCV viral loads, a one-step real-time RT–PCR assay using Lightcyler has been established. The nucleotide sequences of 5′-UTR regions of HCV isolates deposited in GeneBank were aligned. Based on the conserved regions, two primers and two probes were designed for the real-time RT–PCR assay. WHO HCV standards in human plasma with known international units were employed for optimizing the cycling conditions of Lightcycler assay and generation of standard curve. The sensitivity of detection of this one-step RT–PCR assay is between 5 and 50 IU (international unit). The optimal Mn^{++} concentration is 3.25 mM. It can amplify all the genotypes tested including 1a, 1b, 2a, 2b, 3a, 4, and 6a. This assay provides a rapid, sensitive and quantitative test to HCV infection.

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Chimeric HCV Replicons and Distinct Cellular Requirements for HCV and BVDV Replicon Replication

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HCV replicon cell lines were generated for several genotype 1b strains, and efficient replication of genotype 1a replicons

was recently achieved by incorporating an S2204I adaptive substitution into NS5A. In this report, we have used a genetic approach to isolate 1a replicon cell lines by gradually increasing selective pressure through establishment of chimeric replicons. These replication competent chimeras have been used to generate stable cell lines that showed high levels of HCV replication and provided information that permitted the generation of a replicon cell line composed entirely of genotype 1a sequence. During this work we found that cured 1a replicon cell lines were better able to support HCV replication than cured 1b cells, suggesting there are important differences in the cellular environments of these cells. In addition, replication of a closely related bovine viral diarrhea virus (BVDV) replicon was much greater in naïve Huh-7 cells as compared to cured HCV cells. This would suggest that the cellular environment that is more permissive for HCV replicon replication appears to be HCV specific. To further compare the cellular requirements for HCV and BVDV replication, a selectable, subgenomic replicon cell line for BVDV was established. Cured BVDV cells were then used to compare the replication ability of HCV and BVDV and the distinct cellular requirements for HCV and BVDV replicon replication determined.

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High-Throughput Specificity HCV Replicon Screening Assay

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A High-Throughput Specificity Screening (HTSS) assay for the HCV replicon is presented. The assay enables the amount of inhibition associated with test compounds to be quantified specifically in a single well format using three individual but compatible assays. The assays are used for quantifying HCV replicon inhibition, the specificity of inhibition relative to Bovine Viral Diarrhea Virus (BVDV) and the amount of toxicity associated with the inhibition. The HTSS assay uses HCV and BVDV replicon cell lines placed in the same well on a test plate. After incubation with test compounds, three assays are used for quantifying viral replicon inhibition specificity relative to toxicity. HCV replication levels are indirectly measured using an essential enzyme found in all HCV replication systems and is based upon specific cleavage of a HCV FRET peptide by the virally encoded NS3 protease. Specificity of the test compounds for HCV is quantified by use of a luciferase reporter gene incorporated into the BVDV genome. The conversion of Alamar Blue to a non-toxic product by cellular enzymes is used as a basis for quantifying the amount of toxicity associated with the test compounds. The use of this assay format is demonstrated by presenting data showing the ability to discriminate between a BVDV specific inhibitor, compound 1453, and a HCV specific inhibitor.

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Screening for Hepatitis C Virus Antiviral Activity with a Cell-Based Secreted Alkaline Phosphatase Reporter Replicon System

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We have developed a phased screening system for discovery of novel compounds with activity against hepatitis C virus (HCV). The primary screening assay utilizes dicistronic subgenomic HCV replicons in which the upstream cistron has been modified to express the human immunodeficiency virus (HIV) tat protein. When these replicons are stably transfected into Huh7-derived cells that express secreted alkaline phosphatase (SEAP) under transcriptional control of the HIV long terminal repeat promoter, there is a good correlation between the abundance of intracellular HCV RNA and the amount of SEAP in the culture medium. Thus, active compounds are easily identified by direct enzymatic quantification of SEAP in the medium without the need for post assay cell processing. Compounds that reduce SEAP activity without causing cellular toxicity are next evaluated in a second Huh7-derived cell line constitutively expressing SEAP under control of the tat-HIV promoter axis, independent of HCV RNA replication. This specificity control allows us to detect compounds that cause reductions in SEAP activity that are not related to suppression of HCV RNA replication. Those compounds that show HCV-specific activity in primary assays then undergo evaluation by real-time RT–PCR to directly quantify reductions in HCV RNA. We have found excellent agreement between the SEAP and RT–PCR assays. This phased system provides an efficient and cost effective screen for identification and characterization of compounds with antiviral activity against HCV. Data will be presented demonstrating the utility of the screening system with a number of compounds.

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Replication-competent Hepatitis C Virus Subgenomic Replicons Containing Engineered Affinity Tags Within the NS5A Protein

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To study viral and cellular proteins in the HCV replication complex, we have modified a HCV Con 1 1b replicon to express an affinity tag sequence within the coding sequence of NS5A. Amino acids comprising two epitopes, 6-His (HHHHHH) and FLAG (DYKDDDDK), were incorporated into a region of the NS5A protein. HCV genomes containing these

inserts were translated and processed as expected. These genomes were also able to replicate in a transient replication system. Using G418 selection, stable replicon cell lines could be isolated which retained the sequences even after multiple passages, and replicated at levels similar to wild-type (wt). Tagged NS5A protein could be isolated using the appropriate affinity reagents under non-denaturing conditions, indicating that the epitopes were accessible. In addition, single tagged and double tagged replicon cell lines have been generated using two different selectable markers. These tagged NS5A proteins can provide affinity column based methods for identifying both host and viral components in the HCV replication complex and to determine whether NS5A forms a dimer.

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Optimisation of Peptide-based Inhibitors of Full-Length Hepatitis C Virus NS3 Protease

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Hepatitis caused by Hepatitis C Virus is a serious health problem worldwide—about 2% of the world population is infected. There is still no etiological treatment available. The bifunctional protease/helicase NS3 protein of HCV is currently a major target for the search of potential anti-HCV drugs. In our work we synthesized and evaluated in vitro inhibition of the full-length NS3 protease and some other serine and cysteine proteases by peptides based on the sequence of the non-prime side of the natural NS3 substrate. Hexa- and pentapeptide inhibitors with three types of residues at C-terminus were designed: (a) α-carboxylate; (b) electrophilic serine trap groups; (c) carboxylic acid bioisosteres. Molecular modelling studies had revealed that high inhibitory potency and selectivity of inhibitors containing acyl sulfonamide group could be explained by specific interactions of the acyl sulfonamide group with the active site of NS3 protease.

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Non-Nucleoside Allosteric Inhibitors of HCV NS5B Polymerase: Novel Benzimidazole Diamide Derivatives with Replicon Activity

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Current therapies for HCV infections, based on combinations of pegylated interferons and ribavarin, are ineffective in a significant proportion of cases and are associated with the occurrence of severe side effects. HCV NS5B polymerase is a central enzyme of the replicative machinery of the virus and has become a target of choice for the screening and design of small molecule inhibitors that, in principle should interfere with viral replication. We recently described the discovery and optimization of benzimidazole 5-carboxylic acid derivatives which are specific inhibitors of this enzyme. The evolution of this class of compounds towards novel diamide derivatives which show activity in a cell-based assay of HCV RNA replication (replicons) will be described. In particular, it will be shown how parallel synthesis was applied to the search for new linkers to connect essential pharmacophores, leading to novel structures with cellular potency and new prospects for further optimization.

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Synergistic Inhibition of Replication of BVDV by PG 301029 Used in Combination with Ribavirin or Ribavirin and Interferon

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At present, therapy for hepatitis C infection includes the combination of ribavirin and interferon-α aimed at reducing viral replication and modifying the immune response of the host. PG 301029 has been discovered to inhibit the replication of BVDV through a novel mechanism involving interference with the synthesis of viral RNA, thereby reducing the level of progeny virions produced from infected cells. The antiviral efficacy and toxicity results presented suggest that effective therapy for hepatitis C infection may include the combination of PG 301029 with interferon or ribavirin, or the addition of PG 301029 to the cocktail of ribavirin and interferon. Our results suggest that the combination of PG 301029 with interferon or ribavirin would synergistically inhibit hepatitis C and that the addition of PG 301029 would yield a significant reduction in the toxicity of ribavirin. Specifically, the combination efficacy and toxicity results obtained with PG 301029 may be summarized as follows: (1) Ribavirin and interferon are used clinically as a combination therapy in HCV-infected patients, but the efficacy of the combination against BVDV in vitro has not been reported. We therefore performed a combination antiviral evaluation of ribavirin and interferon and found the combination of agents to exhibit a significant synergistic anti-BVDV activity in our in vitro system, reflecting the clinical experience with this combination. (2) The combination of PG 301029 with ribavirin does not show either a significant synergistic or antagonistic effect on anti-BVDV activity. Interestingly, the combination of PG 301029 with ribavirin resulted in a significant reduction in the toxicity of ribavirin in the target cells. Thus, the addition of PG 301029 may expand the therapeutic antiviral index of these two agents when used together. (3) Combination of inter-

feron with PG 301029 resulted in a moderately synergistic effect on anti-BVDV activity in the absence of toxicity. (4) Evaluation of the antiviral effects of the combination of all three compounds, suggest that no additional synergistic antiviral activity was observed upon addition of PG 301029 to the mixture of ribavirin and interferon (additive effect).

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In Vitro Combination Studies of VX-950 (a HCV Protease Inhibitor) or VX-497 (an IMPDH Inhibitor), Two Novel Anti-HCV Clinical Candidates, with IFN-α

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The standard of care for patients with HCV is pegylated IFN-α plus ribavirin which results in a sustained response (SR) in less than 50% of genotype 1 patients after 48 weeks of treatment and is associated with anemia and flu-like symptoms. More efficacious treatment regimens are needed to increase the SR rate and decrease the length of treatment. We have adopted a multi-prong strategy to develop new anti-HCV agents. One approach is to use structure-based drug design (SBDD) to directly target essential viral enzymes such as the NS3·4A protease. This has led to the discovery of VX-950, our first HCV protease inhibitor clinical candidate. VX-950 inhibits HCV RNA replication in HCV replicon cells in a time- and dose-dependent fashion. Conditions have been identified in which treatment with VX-950, alone or in combination with IFN-α was able to completely clear the HCV RNA and where no rebound was observed after withdrawal of the inhibitor. A second approach is to target a cellular enzyme, such as inosine 5′-monophosphate dehydrogenase (IMPDH). Although the exact mechanism of action by which ribavirin increases the SR rate is unclear, ribavirin is thought to reduce the proliferation of a number of viruses by inhibiting IMPDH. VX-497, a more potent IMPDH inhibitor, is currently in clinical trials for hepatitis C. The effect of VX-950 or VX-497 in combination with IFN-α on viral RNA clearance in the HCV replicon cells will be discussed.

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Clinical Evaluation of a Human Monoclonal Antibody against the Envelope Protein (E2) of HCV for Prevention of HCV Infection

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Purpose: To develop an effective therapy to prevent HCV infection in liver transplant patients. **Methods:** Human monoclonal antibodies were generated from peripheral blood B cells isolated from individuals infected with HCV genotype 1b. These antibodies are directed against the HCV envelope protein (E2) and have high affinity and broad reactivity against different genotypes. They were shown to neutralize HCV in vitro in a cell-based infection assay and in vivo in the HCV-Trimera mouse model. HepeX-C, a single human monoclonal antibody, was further developed and studied clinically for safety, tolerability, and potential antiviral activity in phase 1 clinical studies in chronic HCV patients. **Results:** In a phase 1a study, single doses of 0.25, 1.0, 2.5, 10, and 40 mg of HepeX-C were administered to a total of 15 chronic HCV patients. Subsequently, a multidose phase 1b study of 10, 20, 40, 80, and 120 mg of HepeX-C in 25 HCV chronic patients was conducted. The patients had varying levels of HCV-RNA (2.6×10^3 to 1×10^6 IU/ml) and endogenous anti-E2 antibodies (5–550 μg/ml). Administration of HepeX-C was well tolerated in all dose groups with no drug-related serious adverse events. Reduction in viral load is infusion related. In phase 1a, transient reductions in HCV-RNA (2- to 100-fold) were observed in 8 out of 15 patients following a single dose infusion. In phase 1b, response was defined as more than 0.5-log reduction in HCV-RNA from pre-treatment baseline levels at one or more time points following administration of HepeX-C. Eight out of 25 patients (32%) had at least 1-log reduction and 18 out of 25 patients (72%) had at least 0.75-log reduction. Different patterns of response and viral kinetics were observed in all dose cohorts. **Conclusions:** The good safety and efficacy data from these trials provided a basis for a phase 2a pilot study for the prevention of recurrent HCV infection in liver transplant patient. A multicenter, blinded, placebo controlled study is currently underway in 24 liver transplant patients receiving 20, 40, 80, 120, 240 and 480 mg doses of HepeX-C.

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Effect of Interferon-Alpha and Interferon-Inducers on West Nile Virus in Cell Culture and in Mouse and Hamster Animal Models

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Studies were conducted in cell culture and in rodent animal models to determine the efficacy of interferon-alpha (IFN-α), IFN inducers and ribavirin alone or in combination with IFN in treating WNV. Injection (i.p.) of IFN-α B/D (q.i.d. × 7 days), Ampligen (every other day × 7) and topically applied imiquimod (q.i.d. × 7 days), administered 1 day before viral challenge were effective in protecting, respectively 100, 100 and 70% of BALB/c mice from mortality induced by subcutaneous injection of WNV. The reduce mortality correlated with reduced plasma viremia. When

IFN-α B/D or Ampligen treatments were delayed to 4–6 h before viral challenge in mice, efficacy was greatly diminished. Infected Syrian golden hamsters treated with Infergen and Ampligen 4–6 h before viral challenge gained more weight and had a greater survival than saline-treated animals. A combination study of subcutaneously administered Infergen (5–0.05 μg/kg/day) and ribavirin (75–7.5 mg/kg/day) in >7-week-old hamsters demonstrated that Infergen was dose-responsive in reducing mortality and disease signs; however, it was not synergistic in its antiviral effects when combined with ribavirin. Ribavirin treatment alone increased mortality of infected hamsters. Future studies to optimize IFN-α treatment and to determine efficacy at times post-viral challenge when animals have clear signs of disease are warranted.

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Prevention of Viral Invasion by Immunocamouflage of Target Cells

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Viral invasion of cells is mediated via specific interactions between viral capsid proteins and cell surface receptors. Current approaches towards preventing these interactions are univalent in that they attempt to block specific receptor–ligand interactions. Consequently, a more universal (i.e., non-specific) method of preventing viral invasion would be advantageous. To this end we have pioneered the use of immunocamouflage of viral target tissues induced by the covalent grafting of methoxypoly(ethylene glycol) [mPEG] to the cell membrane. The grafted mPEG creates a nontoxic physical barrier that excludes larger particles (e.g., viruses) while allowing small molecules (e.g., amino acids) to readily pass. To determine the efficacy of immunocamouflage in preventing viral invasion, we have utilized a Simian Virus 40 (SV40) model system. In this model, target cells (CV-1) were covalently modified with variable amounts of activated cyanuric chloride mPEG (cmPEG; 0–15 mM mPEG; 5 kDa) prior to SV40 exposure. Importantly, cmPEG-modification had no adverse effects on cell viability. As hypothesized, cmPEG modified CV-1 cells demonstrated significantly ($P < 0.001$) reduced rates of infection. Using a multiplicity of infection of 0.5, approximately half of the control (unmodified) CV-1 cells (2858 positive/6765 total) were infected at 24 h as evidenced by T antigen staining. In contrast, at concentrations as low as 0.6 mM cmPEG, only 8% of the CV-1 cells were T antigen positive (642/7559) at 24 h. Prolonged incubation for up to 72 h confirmed the cmPEG protection. By 72 h, 93% (6729/7245) of the control cells were infected in contrast to 27% (2146/7806) of cmPEG-modified (0.6 mM) cells. The immunocamouflage effect was dependent upon the covalent grafting of the cmPEG as soluble PEG (15 mM) afforded no protection against SV40 infection (46% versus 43% T antigen positive at 24 h). in sum, these data suggest that the covalent modification of target cells and tissue (e.g., nasopharyngeal surfaces) with activated mPEG could provide a potent, multivalent, means of prophylactic protection against a wide range of viral infections.

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Effects of an HIV-1 Attachment Inhibitor on the Conformation of the Viral Envelope Gp120

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BMS-378806 is a prototype HIV-1 attachment inhibitor with good antiviral and pharmacokinetic properties. It binds specifically to gp120 and prevents gp120/CD4 interactions. To extrapolate the inhibition mechanism and to better understand its impact on gp120 conformation, BMS-378806 and gp120 interactions were first investigated by deleting the variable loop(s) and probing with thrombin and anti-gp120 antibodies. Deletion of loops V1, V2 and V3 results in a core gp120 defective in BMS-378806 binding. Additional studies showed that the V3 loop is essential, while loops V1 and V2 display an important, but reduced role in BMS-378806 binding. The role of V3 in BMS-378806-binding to gp120 was evaluated by probing with thrombin. The binding of BMS-378806 reduced the accessibility of the V3 loop to proteolytic cleavage, while sCD4 actually enhanced cleavage. These results suggest a distinct BMS-378806-induced structural change in gp120. Studies with anti-gp120 antibodies (A32, 15e, 17b, 3.9F, and C11) revealed that BMS-378806 inhibits gp120 binding to 17b (recognizing overlapping epitopes with CCR5) in a dose dependent manner, suggesting conformational changes also occurred around the CCR5 binding site(s). BMS-378806 mediated conformational changes on gp120 could confer potential changes in the CD4 binding pocket of gp120, contributing to the exclusion of CD4 docking. Furthermore, conformational changes affecting the CCR5 binding region may reduce the efficiency of viral entry process and increase virus susceptibility to other entry inhibitors.

Oral Session III: Mini-Symposium—What's New in Emerging Infections

No abstracts.

Oral Session IV: Herpesvirus, Poxvirus Infections

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Differing SARs and MOAs Emerge for the Highly Potent Anti-VZV, BCNAs, and Their Anti-HCMV dd Analogues

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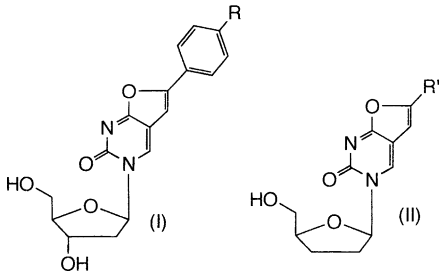
Our laboratories recently discovered the exquisitely potent (<1 nM) and selective (SI > 1,000,000) anti-VZV BCNAs of general structure (I). Optimal activity was noted for a *p*-pentyl chain on the aryl ring. We now note that alkenyl chains may substitute in the *p*-position with sub-nanomolar activity, that alkynyl chains lead to a loss of activity, and that *meta* and, particularly, *ortho* isomers are vastly less active (>20,000-fold).



In sharp contrast, we report that aryl substitution of the alkyl chain R′ in our anti-HCMV dideoxy nucleosides (II) (R′ = pPhR) leads to a loss of activity. This further supports the view that the anti-VZV and HCMV compounds operate by quite discrete MoAs.

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Synthesis and Antiviral Activity of Certain Chlorinated Indole Nucleosides as Potent and Selective Inhibitors of HCMV Replication

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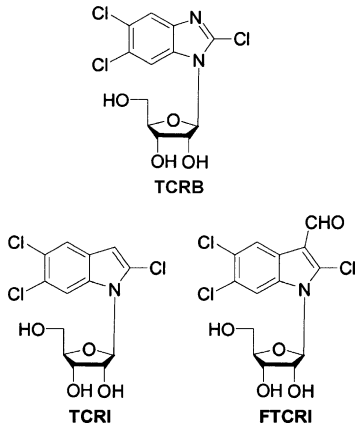
The benzimidazole nucleoside analog TCRB is a potent and selective inhibitor of HCMV replication in vitro, but is rapidly degraded in vivo, limiting its potential as a therapeutic agent. Indole nucleosides should be much more stable to glycosidic bond cleavage due to the much lower pK_a of the heterocycle. Although the analogous trichloroindole riboside TCRI was inactive against HCMV replication, several analogs containing hydrogen bond-accepting substituents at the 3-position, such as the 3-formyl analog FTCRI, have been found to be potent and selective inhibitors of HCMV replication. The 3-substituted analogs were synthesized by glycosylation of the chlorinated heterocycle, followed by electrophilic addition at the 3-position. SAR for this series of compounds indicates that a hydrogen bonding substituent at the 3-position is required for activity, but that different modifications of the sugar are well tolerated with regard to antiviral activity.

Acknowledgments: These studies have been supported by NIAID grant U19-AI31718 and NIH grant T32-GM07767.



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Comparative Antiviral Activity of Alkyl and Alkoxyalkyl Esters of Cidofovir Against HCMV Infected Cells, In Vitro

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We reported previously that alkoxyalkyl esters of cidofovir (CDV) are highly active and selective against herpes viruses and orthopoxviruses in vitro and in vivo. The alkoxyalkyl esters of CDV were designed to mimic alkylglycerophospholipids and to be able to undergo metabolic cleavages common to this class of natural compounds. To evaluate the importance of the oxygen heteroatom two to three carbons distant from the phosphonate moiety, we synthesized a series of CDV esters lacking the oxygen and compared their activity with that of alkoxyalkanol esters. HCMV (AD-169 strain) was used to infect HFF cells; drugs were applied and EC_{50} values determined by plaque reduction. Unsaturated long chain alkyl esters of CDV were highly active against HCMV with an optimal chain length of 20–22 carbon atoms. EC_{50} values for the most active compounds ranged from 3 to 7 nM. When the alkoxyalkyl esters of CDV are compared as a function of the number of atoms beyond the phosphonate, the most active members of the family are slightly more active than the alkyl-CDVs, with 20 and 22 atom analogs having EC_{50} values of 0.9–1.5 nM against HCMV. The most active compound against HCMV is oleyloxypropyl-CDV, EC_{50}

0.15 nM. The alkoxyalkyl-CDV analogs have been shown to be orally bioavailable but it is not presently known if this is also the case for the straight chain alkyl esters of CDV. In summary, alkyl esters of CDV are highly active and selective inhibitors of HCMV with EC_{50} values in the nanomolar range.

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Identification of the Mode of Action of Methylenecyclopropane Analogs using a Chemotypic Clustering Analysis

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Methylenecyclopropane analogs are a relatively new class of compounds of which some members are potent inhibitors of cytomegalovirus infection in vitro and in animal models. Although it has been postulated that these compounds are inhibitors of DNA synthesis, their exact mechanism of action is not well defined. The purpose of these studies was to determine the mode of action of this class of compounds and in particular a lead compound, ZSM-I-62, by assessing its effect on viral DNA synthesis and the expression of viral gene products. ZSM-I-62 strongly inhibited viral specific DNA synthesis at concentrations that did not induce detectable levels of cellular toxicity. Consistent with these results, immediate early and early gene expression were relatively unaffected in cells treated with this compound, whereas late gene expression was significantly reduced. The susceptibility of a number of recombinant viruses with engineered deletions in viral open reading frames was also determined to help identify viral proteins that play a role in the mechanism of action of this molecule. Of the viruses tested, only recombinant viruses with deletions in *UL97* were resistant to the antiviral activity of ZSM-I-62. The deletion of this open reading flame reduced the EC_{50} a 100-fold and demonstrated that this gene is required for the activity of this compound, presumably for the initial phosphorylation of the drug to the level of the monophosphate. Taken together, these data are consistent with a mechanism of action that is similar to ganciclovir where *UL97* is required for the phosphorylation of the drug and the active triphosphate form of this compound is a potent inhibitor of viral DNA synthesis.

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Effect of Oral Treatment with a Methylenecyclopropane Analog, ZSM-I-62, on Replication of Human Cytomegalovirus (HCMV) or Murine Cytomegalovirus (MCMV) Infection in Animal Models

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Human cytomegalovirus (HCMV) can cause a wide variety of clinical manifestations in immunocompromised hosts or transplant recipients. Two models using severe combined immunodeficient (SCID) mice implanted with human fetal tissue and subsequently infected with HCMV and BALB/c mice infected with MCMV were used to evaluate efficacy of the methylenecyclopropane analog, ZSM-I-62. In one HCMV model, human fetal retinal tissue was implanted into the anterior chamber of the mouse eye and inoculated 8–12 weeks later with 8000 pfu of the Toledo strain of HCMV. In the second model, human fetal thymus and liver (thy/liv) tissue were implanted under the kidney capsule of mice and inoculated 16–20 weeks later with 4700 pfu of HCMV. In both models tissue samples were obtained at various time points for quantitation of HCMV titers by plaque assay. In general, replication of the Toledo strain of HCMV in the implant tissue increased through 21–28 days and then gradually decreased to undetectable levels by 8 weeks post infection. To determine the efficacy of ZSM-I-62, oral treatment with vehicle or 45 or 15 mg/kg of ZSM-I-62 was initiated 24 h after infection and continued for 28 days. Ganciclovir (GCV) at 45 mg/kg was administered i.p. daily as a positive control. Results in both HCMV models indicated that ZSM-I-62 was highly effective in reducing replication to undetectable levels compared to the vehicle control. The effect of ZSM-I-62 was greater than GCV at most time points. In MCMV infected mice, ZSM-I-62 was highly effective in preventing mortality when administered orally at 50, 16.7, 10, 5.6, 3 or 1 mg/kg beginning 24, 48 or 72 h post viral inoculation. The effect of ZSM-I-62 given orally once a day at 10 mg/kg beginning 24 h after infection on the pathogenesis of MCMV infection was also evaluated. These results indicated a 4-$\log_{10}$ reduction in virus titers in liver samples by Day 3 post infection and a 3.5-$\log_{10}$ reduction in spleen samples by Day 7. These data indicate that the methylenecyclopropane analog, ZSM-I-62, was highly efficacious in these three animal models and should be evaluated for use in HCMV infections in humans.

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Inhibition of Orthopoxvirus DNA Polymerases by Cidofovir Diphosphate: In Vitro Enzymatic Studies Using Highly Purified Vaccinia Virus DNA Polymerase

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Cidofovir (CDV) and its alkoxyester derivatives show promise as therapeutic agents for the treatment of Orthopoxvirus infections including human smallpox. Vaccinia virus DNA polymerase shares 98% amino acid identity with variola (smallpox) DNA polymerase and we have been examining the effect of the active intracellular metabolite, CDV diphosphate (CDVpp), on DNA synthesis catalyzed by recombinant vaccinia polymerase. These studies show that vaccinia DNA polymerase can use CDVpp as a substrate analog of dCTP ($K_{M,dCTP} = 2.2\,\mu M$), incorporating CDV opposite a G in the template strand. The resulting 3′-CDV terminated oligonucleotide primer still appears to serve as a substrate for the enzyme, but the presence of CDV then causes a chain termination event, immediately following the addition of the next dNMP. This enzymatic behavior resembles the effect of CDVpp on chain extension catalyzed by cytomegalovirus DNA polymerase, except that the herpes polymerase is reported to promote chain termination opposite two template G's, whereas vaccinia virus polymerase seems to terminate synthesis opposite any 3′-GX-5′ motif (where "X" is any nucleotide). The cause of this effect is uncertain although it may be due to some CDV-induced instability or disorder located near the 3′ hydroxyl of the growing DNA chain. We are currently contrasting these data with the effects on DNA chain extension caused by related acyclic nucleotide phosphonate diphosphates, as well as examining what effect varying the primer template sequence has on the efficiency and site of chain termination.

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A Cidofovir-Resistant Form of the Highly Virulent WR Strain of Vaccinia Virus is Cross-Resistant to Related Antiviral Agents and is Highly Attenuated for Virulence in Mice

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The wild-type (WT) vaccinia (WR strain) virus is highly virulent to mice when inoculated by intranasal route. Parenteral treatments with cidofovir (CDV, HPMPC) can prevent death. After 15 passages in Vero cells in the presence of increasing concentrations of CDV, a CDV-resistant (CDV-R) virus was produced and compared to WT virus. In plaque assays, CDV inhibited WT virus in Vero cells by 50% at 60 μM compared to inhibition of the CDV-R virus at 790 μM. In a virus yield reduction assay, the 90% inhibitory concentration of CDV was 45 μM for WT virus and 420 μM for the resistant virus. Cross resistance was seen with HPMPA, HPMPG, and HDP-CDV, but not with ribavirin and 2-amino-7-[(1,3 dihydroxy-2-propoxy)-methyl]purine. The resistant virus produced 1000-fold less infectious virus than did WT virus in mouse C127I cells, yet both viruses replicated to similar titers in Vero (monkey) cells. In mice the CDV-R virus was attenuated for virulence, producing no deaths at 5×10^6 infectious particles (PFU) per mouse. The WT virus was virulent below 10^5 PFU per animal. Because death could not be used as an end point for antiviral studies with the CDV-R virus in mice, we used lung and snout virus titer reductions as efficacy parameters. CDV, HDP-CDV, adenine arabinoside (ara-A), and ribavirin treatments were unable to reduce lung virus titers even three-fold. Snout virus titers were reduced over 10-fold by ara-A, CDV, and HDP-CDV treatments. These experiments indicate that development of vaccinia (WR strain) virus resistance to CDV leads to attenuation of virulence in mice, which is related to reduced ability of the virus to replicate in mouse cells. Infection parameters besides death can be used to study effects of antiviral agents.

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Successful Cidofovir Treatment of Smallpox-Like Disease in *Variola* and Monkeypox Primate Models

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The extent of the characteristic pox rash of smallpox and monkeypox correlates with disease severity in humans. Intravenous infection with 10^7 PFU of monkeypox produces a lethal model that faithfully reproduces the rash lesional disease characteristic (>500 lesions) of smallpox and monkeypox, while 10^8 PFU *Variola* produces a similar lesional disease (WHO category "grave") and 33% mortality in cynomolgus monkeys. Intravenous infection bypasses the incubation period, resulting in rapid development of high levels of virus and rapid disease onset. Cidofovir prophylaxis completely protected both models with no signs of illness and control of viral replication in blood, while the placebo-treated animal had >850 lesions and levels of virus in blood $> 10^7$ genomes/ml with one of three dying on day 12. Cidofovir treatment as late as 48 h after infection re-

duced viral load and lesion counts. Doubling the cidofovir dose resulted in significantly better control of viral replication. Comparing the models showed that viral replication occurred more rapidly in the *Variola* model with 10-fold higher peak genome levels in blood, suggesting a more severe model. These two models taken together should provide appropriate, although severe, models to meet the FDA Animal Efficacy Rule for evaluation of drug efficacy.

Oral Session IV: Herpesvirus, Poxvirus Infections

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Inhibition of SARS Coronavirus In Vitro by Human Interferons

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Severe acute respiratory syndrome (SARS) is a respiratory illness caused by a coronavirus, the SARS-associated coronavirus (SARSCoV). It is a life-threatening and highly contagious, febrile respiratory illness that was initially described in early 2003. Treatment for the disease is supportive as there are no approved or universally recommended therapies for SARS. Using cytopathic effect (CPE) reduction assays that were verified spectrophotometrically by neutral red (NR) uptake assay of the same plate, we evaluated a number of interferons for anti-SARSCoV activity in African green monkey kidney cells. Some interferons were also evaluated in virus yield reduction assays. All of the human recombinant alpha interferons tested were inhibitory to SARSCoV replication, with EC_{50} values $\leq$50 units/ml. Human leukocyte-derived interferon alpha-n3 was active down to 32 IU/ml. By virus yield reduction, that same interferon had an EC_{90} = 32,000 IU/ml. This result may reflect the fact that virus cytopathic effect was detected at all dilutions of every interferon tested, albeit at reduced levels at higher concentrations of compound. Mouse interferon alpha A was not inhibitory up to 5000 IU/ml by visual assay. An interferon beta la was also evaluated and had an EC_{50} = 0.8 μg/ml. These data suggest that both alpha and beta interferons may be potentially useful therapies for treating SARS.

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Inhibitory Activity of Vancomycin, Eremomycin and Teicoplanin Aglycon Derivatives Against Feline and Human (i.e. SARS) Coronaviruses

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Recently, aglycon derivatives of the antibiotics vancomycin, eremomycin and teicoplanin that are further modified by the introduction of lipophilic groups, have been reported to inhibit the replication of human immunodeficiency virus in cell culture [Balzarini et al., J. Med. Chem. 46 (2003) 2755–2764]. We have now evaluated a wide variety of these antibiotic analogues against feline coronavirus (strain FIPV-1146)- and human (SARS) coronavirus (strain Frankfurt-1) (SARS-CoV)-induced cytopathicity in Crandell feline kidney cells (Crfk) and Vero cells, respectively. Several compound derivatives were shown to inhibit both viruses at concentrations ranking between 1 and 100 μM, depending on the nature of the test compound and its (lipophilic) substituents. There was no strong correlation between the inhibitory potency of the test compounds against both viruses. In most cases, the inhibitory activity of the test compounds was more pronounced against the feline coronavirus than SARS-CoV. However, in several cases, activity was found against the feline coronavirus but not the SARS-CoV and, vice versa, some compounds were exclusively inhibitory against the SARS-CoV but not FIPV. Time-of-addition studies revealed that the aglycon antibiotic derivatives may act at an early step (i.e. virus entry) of the (feline) coronavirus infection cycle.

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In Vitro Investigation of Potential Therapeutics for the Severe Acute Respiratory Syndrome (SARS)

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The severe acute respiratory syndrome (SARS) was detected in Guangdong, China, in November 2002. The disease spread around the world leading to 8098 suspected cases and 774 deaths in 27 countries. The SARS-associated coronavirus (SARS-CoV) was identified to be the infectious agent causing SARS. This report describes the in vitro investigation of drugs considered for SARS treatment. Glycyrrhizin inhibits SARS-CoV replication in vitro. Moreover, first clinical trials were promising. The mechanism of anti-SARS activity of glycyrrhizin remains to be determined. Presumably, increased nitric oxide (NO) production due to induction of inducible nitric oxide (NO) synthase expression plays a role, since SARS-CoV replication is highly sensitive to inhibition by NO. The broad-spectrum antiviral drug ribavirin was chosen for the treatulent of SARS-patients even before the SARS-CoV had been identified. Although clinical results are judged contradictory and ribavirin was initially found to be non-effective in vitro, our new results demonstrate ribavirin to inhibit SARS-CoV replication in MA-104 and Caco-2 cells. The reason for these differences may be insufficient phosphorylation and activation of ribavirin in Vero cells. Between the interferons, only interferon-β inhibited SARS-CoV replication in clinically relevant concentrations in vitro. Corticoids were used to con-

trol cytokine storm during SARS pathology. Therefore, influence of hydrocortisone on inflammatory molecules was determined in a SARS-CoV-permissive human cell culture model (Caco-2). High concentrations of hydrocortisone reverted SARS-CoV-induced up-regulation of proinflammatory genes and prevented activation of proinflammatory transcription factors. These results show, that glycyrrhizin, ribavirin, interferon-β, and hydrocortisone might have a role in future treatment regimens for SARS, although risk–benefit analysis has to be performed carefully.

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Fusogenic Mechanism of SARS-Associated Coronavirus

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Severe acute respiratory syndrome (SARS), caused by SARS-associated coronavirus (SARS-CoV), is an emerging disease which has threatened the public health and economy worldwide. The mechanism by which SARS-CoV fuses with target cells is very important for developing therapeutics and prophylactic agents against SARS-CoV. We designed and synthesized two sets of peptides corresponding to the sequences of the SARS-CoV S protein heptad repeat 1 and 2 (HR1 and HR2) regions. We tested the inhibitory activity of these peptides against SARS-CoV infection and found that only one peptide derived from the HR2 region, designated CP-l, inhibited SARS-CoV infection at μM level. We also investigated the interactions between the HR1 and HR2 peptides using a series of biophysical techniques, including surface plasmon resonance, sedimentation equilibration analysis, circular dichroism, native polyacrylamide gel electrophoresis, size exclusion high performance liquid chromatography, and computer-aided homology modeling and molecule docking analysis. CP-1 bound, with high affinity, to another peptide derived from the HR1 region, named NP-1. CP-1 alone had low α-helicity and self-associated to form a homologous trimer. Mixing CP-1 with NP-1 at equimolar concentrations resulted in formation of six-helix bundle, similar to the fusogenic core structure of the HIV-1 gp4l. These results suggest a fusogenic model of the SARS-CoV. After SARS-CoV binds to the receptor, such as ACE2, the S protein may change conformation by association between the HR1 and HR2 regions to form an oligomeric structure, leading to fusion between the viral and target cell membranes. The peptide CP-1 may bind to the HR1 region at the prefusion intermediate state and interfere with the S protein conformational changes, resulting in inhibition of SARS-CoV fusion with the target cells.

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Towards a Model for Small Molecule Inhibition of Virus Fusion: Photoaffinity Labeling Identifies the Binding Pocket of an RSV Inhibitor

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During virus entry, the respiratory syncytial virus (RSV) fusion protein undergoes a series of conformational rearrangements into a trimer-of-hairpins structure that results in the apposition of viral and cellular membranes. Recently, the small molecule fusion inhibitor BMS-433771 was identified which specifically inhibits RSV fusion in vitro and is orally efficacious in the mouse model of infection. A radiolabeled photoreactive analog of this compound was prepared and used to probe its interaction with native virus. The analog specifically reacted with the F1 subunit of the native protein in a region containing the N-terminal heptad repeat peptide. Under certain conditions, the photoreactive compound was also able to specifically react with a synthetic 42-amino acid peptide containing this heptad repeat. This allowed for precise identification of the amino acids within the F1 that reacted with the probe. The results show that these fusion inhibitors bind within a hydrophobic cavity formed on the surface of the N-terminal heptad repeat trimer that, after the conformational change, is occupied by key amino acid residues on the C-terminal heptad repeat. Therefore, this inhibitor series may prevent the proper hairpin formation required for membrane fusion.

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A Novel Respiratory Syncytial Virus Inhibitor

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Respiratory syncytial virus (RSV) is the cause of one-fifth of all lower respiratory tract infections worldwide, and is increasingly gaining recognition as representing a serious threat to patient groups with poorly functioning immune systems. We have identified a novel series of RSV inhibitors from screening a subset of the Arrow library in an in vitro whole cell infection assay. Extensive SAR analysis has lead to a series of compounds displaying anti-viral activities ranging from 0.4 to >50 μM. Further lead optimisation resulted in the selection of a number of compounds with excellent submicromolar activity against both A- and B-subtypes of RSV in ELISA and plaque reduction assays.

These compounds are approximately 30-fold more active in vitro than ribavirin, the only anti-viral drug currently licensed for the treatment of an RSV infection. Activity has also been displayed against clinical isolates. Furthermore, the compounds showed little in vitro growth inhibition across three human cell lines. Lead compounds were also shown to have a desirable pharmacokinetic profile. Preclinical evaluation of A-60444 has demonstrated excellent safety and Phase I clinical trials are anticipated in the near future.

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Effect of Conjugated Linoleic Acid on Experimental Rhinovirus Illness

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Conjugated linoleic acids (CLA) are naturally occurring dietary lipids and also used as dietary supplements; they have reported in vivo effects on both innate and adaptive immune responses, including reductions in pro-inflammatory cytokine responses. One commercial CLA product (Safflorin^{TM}) reduced lung viral levels and lethality due to experimental influenza infection of rats. Since human rhinovirus (HRV) infections are associated with pro-inflammatory cytokine responses, we tested whether CLA might modulate experimental HRV infection. In a double-blind trial 50 volunteers susceptible to HRV-39 were randomized to oral CLA 2 g per day or placebo (P) for 4 weeks before and 4 days following HRV inoculation. Primary endpoint for the study was the frequency of colds. **Results:** Five subjects (3p, 2 CLA) were excluded from efficacy analysis because of non-HRV-39 infection prior to challenge. HRV-39 infection was documented in 92% of P and 86% of CLA recipients, and no differences in viral shedding were observed. Overall 10/24 P (42%) compared to 7/21 CLA (33%) had developed colds (P = NS). Total symptom scores for the five post-inoculation days tended to be lower with CLA (mean $\pm$ S.D., 7.1 ± 7.1) compared to P (10.9 ± 11.2) (35% reduction). CLA did not reduce nasal symptoms but was associated with highly significant reductions in cough (0 ± 0.2 versus 0.9 ± 2.9) and sore throat (0.8 ± 1.3 versus 2.9 ± 3.6) (72% reduction). Only 1 CLA compared to 7 (29%) P recipients ($P = 0.037$) developed sore throat of moderate or greater severity. CLA was generally well tolerated; no important changes in fasting lipids or glucose were noted, although CLA recipients had significantly lower fasting insulin and leptin levels compared to P. **Conclusion:** These results are the first to indicate that CLA dietary supplementation may have beneficial effects in an acute HRV illness. Studies of nasal cytokines are in progress to determine whether the reductions in sore throat and cough might relate to effects on host inflammatory responses.

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Efficacy of PMEG [9-(2-Phosphonylmethoxyethyl)-guanine] and its Prodrug cPr-PMEDAP [9-(2-Phosphonylmethoxyethyl)-*N*6-Cyclopropyl-2,6-Diaminopurine in Organotypic Cultures of Normal and Papillomavirus ((HPV)-Positive Keratinocytes

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We have recently developed organotypic co-cultures of primary human keratinocytes (PHKs) isolated from neonatal foreskins and the cervical carcinoma cell line SiHa (HPV-16 positive) to evaluate the selectivity of cidofovir, an acyclic nucleoside phosphonate analogue (ANPs) that proved efficacious in the treatment of different clinical manifestations of HPV-induced epithelial cell proliferation. We have now used this system to determine the efficacy and selectivity of other ANPs, i.e. PMEG and cPr-PMEDAP, with potential activity against HPV. In the organotypic raft culture normal keratinocytes stratify and fully differentiate in a manner similar to the normal squamous epithelial tissues, while HPV-positive cell lines exhibit dysplastic morphologies similar to (pre)neoplastic lesions seen in vivo. SiHa cells and normal PHKs were seeded at a 1:1 ratio on top of the dermal equivalent and remained submerged for 48 h. The collagen rafts were raised (day 0) and placed on stainless steel grids, at the interface between air and liquid culture medium. Epithelial cells were then allowed to proliferate for 10 days in the presence or absence of the compounds. After 10 days all cultures were fixed, paraffin-embedded, sectioned and stained with hematoxylin and eosin. In control untreated co-cultures, rafts showed regions with dysplastic morphology, normal epithelium and areas with mixtures of both types. In contrast, rafts that were treated with PMEG (0.5 μg/ml) and cpr-PMEDAP (5 μg/ml) at day 3 post-lifting and with PMEG (0.5 and 5 μg/ml) and cpr-PMEDAP (5 μg/ml) at day 6 post-lifting showed areas of fully differentiated normal epithelium and destruction of the tumor cells. Inhibition of SiHa cell proliferation by both drugs was concentration- and time-dependent. These results point to a selective inhibition of HPV-positive cell growth by PMEG and cPr-PMEDAP.

Poster Session II: Herpesvirus, Poxvirus, Respiratory Virus, and Other Infections

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A Novel Compound IC977467 With Potent Anti-Herpesvirus Activities

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We screened our small molecule library for inhibitors of a physiologically derived herpes simplex viral DNA–protein complex. One of the inhibitors, IC977467, shows potent antiviral activity against several herpes viruses in cell infectivity assays including herpes simplex viruses (HSV-1 and HSV-2), Varicella-zoster virus (VZV), cytomegalovirus (CMV), and Epstein-Barr Virus (EBV). 1C977467 most potently inhibited replication or virally induced cell killing by VZV and CMV followed by moderately potent activity against MSV1, HSV2 and EBV. When compared side by side, IC977467 was equally efficacious against acyclovir-sensitive and acyclovir-resistant clinical isolates as well as a laboratory strain of HSV2. Activity of VZV (reference strain Ellen) was evaluated using quantitative detection of VZV DNA copy numbers with a real-time PCR assay (Lightcycler). Activity of CMV (reference strain AD 169) was evaluated using a virus yield reduction assay. Activities against HSV1 and HSV2 were measured using a cytopathic effect (CPE) inhibition assays. Lastly, activity against EBV (using EBV-producer cell line, P3HR-1) was determined by measuring EBV DNA levels using a real-time PCR assay. IC977467 is slightly toxic to Vero cells at the highest concentration tested (100 μM). We conclude that the antiviral activity of 1C977467 was efficacious against a broad spectrum of herpes viruses.

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A Flow Cytometric Study on Antiviral Effect of Seaweed Polysaccharides against Herpes Simplex Viruses

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Many seaweed polysaccharides have been shown to possess antiviral activity against a variety of animal viruses. In this study, polysaccharides were extracted from the seaweed *Hydroclathrus Clathratus* in Hong Kong by hot water, dilute acid and alkali. The antiviral activities against herpes simplex virus (HSV) of these three kinds of polysaccharides were determined by the cytopathic effect (CPE) inhibition assay and plaque reduction assay (PRA). The alkali extract showed better effect against HSV than the acidic portion but both were less active than the water extract. After the water extract was further purified, an antiviral substance was obtained. It was a polysaccharide with MW about 3938 kDa determined by HPLC. This polysaccharide was tested for its antiviral efficacy against HSV-l and HSV-2 utilizing flow cytometry assay system. The 50% inhibition ratio (IC_{50}) was 8.2 and 5 μg/ml, respectively in Vero cell line with very low cytotoxicity. The inhibition effect was dose-dependent. The results showed that flow cytometry can be used to screen and efficiently evaluate antiviral agents against HSV and that the results were comparable to those obtained by CPE and PRA methods. The antiviral activity of the polysaccharide was mainly due to its effect on virus-cell binding or virus-cell fusion because the compound must be present during virus adsorption or during the whole infection period. The polysaccharide also showed some effect against the penetrative action of HSV into the Vero cells.

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Oligonucleotides Containing the Cognate Recognition Sites for the HSV Transcriptional Regulators ICP4 and α-TIF Reduce the Expression of Target Viral Proteins and Progeny Virus Production

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Herpes Simplex Virus (HSV) promotes the expression of viral proteins through the production of transcriptional factors encoded in the viral genome and via the recruitment of host transcriptional regulators. Viral encoded transcription factors include the virion-associated protein, α-*trans*-inducing factor (α-TIF), and the immediate early gene product, infected cell protein number 4 (ICP4). We hypothesized that cells transfected with oligonucleotides containing binding sites for ICP4 and α-TIF would be protected from viral infection by virtue of competition of the oligonucleotides with the HSV genome for binding to the viral transcription factors. The effect of transfected oligonucleotides containing the cognate-binding sites for either α-TIF or ICP4 on target viral protein expression and on production of infectious viral progeny was assessed in cultured Vero cells infected with HSV. The ICP4-binding site specific oligonucleotide, but not the scrambled ICP4 oligonucleotide, resulted in a significant decrease in the level of glycoprotein D expression, and a concomitant reduction in the production of infectious virus. Similarly, the α-TIF-binding site specific oligonucleotide, but not the scrambled α-TIF oligonucleotide, caused a decrease in both ICP4 and ICP0 expression as well as a de-

crease in the production of infectious virus. These data validate ICP4 and α-TIF as therapeutic targets and demonstrate the utility of oligonucleotides containing either ICP4 or α-TIF specific binding sites as potential treatments for HSV-associated diseases.

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Inhibitors of Herpes Virus and Inflammation

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It is known that keratitis is a lessions in a cornea of the eye set off by herpesvirus-1 and accompanies with cytokines production including interleukins IL-2, IL-1-alpha, IL-12. An influence of cytokines on the cell membrane increases its permeability and may promote virus passing. This supposition is supported by the fact that expression of HSV-1 in vitro is increased by prostaglandins E_1 and E_2. IL-10 and IL-12 controlling an inflammation have antiherpes activity. Data mentioned above give a possibility to suggest that herpes virus may initiate an inflammatory process and cytokines accelerate an infication. Perhaps HSV-1 and inflammation mediators contain resemble fragments in their structure. If it is true that a substance with antiflogistic properties may inhibit HSV-1 replication and vice versa HSV inhibitors may display anti-inflammatory activity. About 10 compounds with different chemical structure which possess both type of activity are described in sources and found dy authors.

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Inhibitors of Herpes Simplex Virus Studied in a Murine Infection Model of the Immunocompromised Host

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HSV is associated with long-lasting and damaging lesions in immunocompromised patients. Antiviral agents control such infections, however, they are prone to resistance. Resistance may be multiple, e.g., aciclovir and foscarnet; more effective strategies to combat HSV in this situation are required. Murine skin infections are good predictors of efficacy of chemotherapy including chronic HSV lesions in the immunocompromised host. In a reproducible model, mice are inoculated by scarification into the skin of the neck. Virus travels via peripheral nerves to the dorsal root and trigeminal ganglionic neurons then to the ipsilateral ear pinna. Skin lesions form a characteristic zosteriform pattern including the ear pinna and quantitative measurements of clinical signs and virus titres can be obtained. Infectious virus in the ear pinna (secondary site of infection) must have passed through the nervous system and may be compared with virus populations in neural tissue and the neck skin (primary site). For immunosuppression, mice are inoculated with cyclosporin-A from two days prior to infection then on alternate days resulting in persistent virus replication for several weeks. Antiviral therapy may be systemic and/or local. We have administered oral famciclovir to yield penciclovir. Sub-optimal doses or discontinuous famciclovir therapy were used to study the effects of therapy on persistent virus replication in the various anatomical compartments and the establishment of latency. The populations of virus in the primary and secondary site were analyzed for the development of drug resistance. The implications for the generation of virus resistance and therapeutic strategies to circumvent resistance in man will be considered.

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Inverse Fourier Transform as a Part of Fractal Microscope in Virus–Cell Interaction Imaging

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We have discussed previously the fractal properties of diffraction patterns obtained by laser irradiation of virus–cell system. It was shown that the diffraction process is equivalent to the direct Fourier transform of the said system's components. We propose here to use the inverse Fourier transform of the target's pattern in order to get the real enlarged image of the viruses attacking the sensitive cell as well as the cell's structural transformation caused by the interaction itself. The set of bright and dark spots, which forms the diffraction pattern, could be coded into set of numbers using the quantification procedure. The full information included into the pattern peaks' diameters and color index is transformed using inverse Fourier technique into set of intersecting bright and dark circles. The full in vitro dynamics of the structural changes of the virus–cell system is described by the changes of circles' diameters and their intersection's area. It was shown, also, that the magnification of the fractal microscope could achieve 10,000× to 100,000×, depending on the laser power. Proposed fractal microscope could be applied as well in vivo experiments until the required magnification will not make us to use projection laser with the output exceeding 10 mW. The fractal microscope based on the inverse Fourier transform could be applied successfully in pharmaceutical antiviral drug design, laboratory and clinical trials of new antiviral preparations.

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Fractal Design of Virus–Cell Dynamic System

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We have shown previously that the fractal approach to the problem of virus–cell interaction gives, at the same time, the unique possibility to obtain and process the data through the sequence of the direct and inverse Fourier transforms. The mathematical apparatus guarantees the mutual exact correspondence between the structural details of virus–cell dynamic (being in permanent change) and their image on the target. The fractal dimension D of the system and that of its image, changing as the object does, remains in the said correspondence range. The laser intensity peaks, which form the speckle pattern of the system under consideration, could be transformed into the hierarchical system of the circles (or squares) according to the choice of the researcher, but conserving the same D value, which depends only on the true intermolecular potential. This potential is responsible for the given structure of the dynamic virus–cell system and the unique, but the typical form of the fractal cluster corresponding both to the system itself and its image as well, processed by computer techniques. The hierarchical fractal design of the virus–cell system, proposed here for the first time, gives the universality, needed for the quantitative description of any possible combination of the virus and corresponding sensitive cell. It should be noted as well that the fractal design of virus–cell dynamic system have all the properties, requested from all the experimental tools of monitoring, including the reliability, reproducibility and preciseness.

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***N*-[(2-Hydroxyethoxy)methyl] Derivatives of Bicyclic Furano and Pyrrolo Pyrimidines and Their Antiviral Evaluation**

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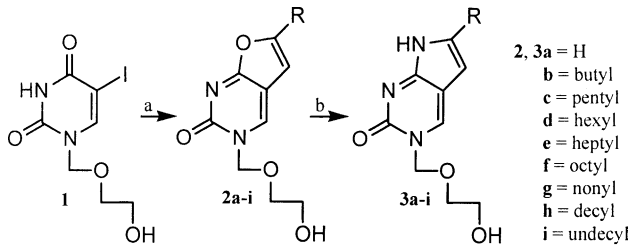
Recently, an entirely new spectrum of antiviral activity was discovered with bicyclic pyrimidine nucleoside analogues (BCNAs). The Baizarini, De Clercq, and McGuigan group found exquisite potency and selectivity of these compounds towards Varicella-Zoster virus (VZV).[1,2] Thus, BCNAs represent a promising class of antiviral drugs.

We have prepared series of acyclic nucleoside analogues of BCNAs with furano (**2**) and pyrrolo (**3**) pyrimidine bases in which the sugar part was replaced by a (2-hydroxy-ethoxy)-methyl moiety (acyclovir analogues). Results of the antiviral SAR studies will be presented.



(a) alkyne/$Pd(PPh_3)_4$/CuI/Et_3N/DMF; (b) NH_3/MeOH.

References:

1a) MeGuigan, C., et al., 2000. Drugs of the Future, vol. 25, pp. 1151–1161.

b) Baizarini, J., et al., 2002. J. Antimicrob. Chemother., vol. 50, pp. 5–9.

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Bicyclic Anti-VZV Nucleosides: Thieno Analogues Bearing Alkylphenyl Side Chain Result in a Reduction in Antiviral Activity

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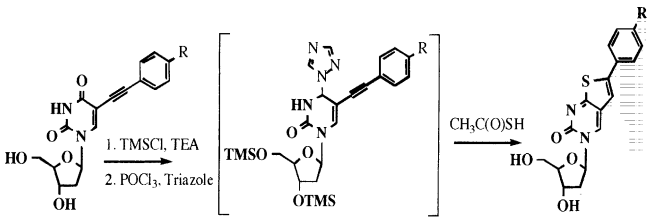
Following the discovery of the 2,3-dihydrofuro[2,3-*d*]-pyrimidine 2-one nucleosides (BCNAs) as a new class of potent anti-Varicella-Zoster Virus (VZV) nucleosides, a number of studies have been made within our group to determine the structure activity relationship (SAR) of these compounds. In particular, replacement of the furo moiety of the parent alkyl bicyclic nucleosides found, that while *pyrrolo* analogues showed a marked decrease in potency, the *thieno* analogues retained full activity against VZV. In further exploration of this work, we herein report the synthesis and biological evaluation of thieno analogues bearing an alkylphenyl side chain. The target structures were prepared from the corresponding 5-alkynyl 2′-deoxyuridine derivatives. The first step involved the protection of the free hydroxyl groups by TMSCI followed by the preparation of C-4 triazole intermediates. Addition of thiolacetic acid resulted unexpectedly in the cyclisation and formation of the desired bicyclic thieno analogues (see Scheme 1 in the following). Biological evaluation of the analogues provided some intriguing results. While the alkyl chain thieno analogues were found to retain full antiviral activity the new thieno analogues bearing an alkylphenyl side chain showed a reduction in antiviral activity against VZV relative to the furano parents with the

p-pentylphenyl analogue 50-fold less active than its furo parent.



Scheme 1- Synthetic pathway for the preparation of thieno BCNAs

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Although the Bicyclic Nucleoside Analogues (BCNAs) are Highly Active Against Varicella-Zoster Virus (VZV), They are Inactive Against Simian Varicella Virus (SVV) Despite Efficient Phosphorylation of the BCNAs by SVV-Encoded Thymidine Kinase

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Simian varicella virus (SVV, *Cercopithecus herpes virus* 7) shares many properties with human varicella-zoster virus (VZV). It causes a natural varicella-like disease in non-human primates. Due to the close similarities between VZV and SVV, SVV infection in non-human primates may be useful as a relevant VZV animal model. We considered the possibility of this monkey model to examine the antiviral efficacy of the bicyclic nucleoside analogues (BCNAs) that, in cell culture, are selectively inhibitory to VZV (EC_{50} in the lower nanomolar range) but not to herpes simplex virus. Whereas the 5-substituted 2′-deoxyuridine derivatives (e.g. BVDU, BVaraU) and the acyclic guanine nucleoside derivatives (i.e. ACV GCV) showed comparable antiviral efficacy against both VZV and SVV in cell culture, the BCNAs were not inhibitory to SVV replication in BSC-1 cells. As phosphorylation by VZV thymidine kinase (TK) appeared to be essential for BCNAs to be antivirally active in VZV-infected cell cultures, we investigated whether SVV TK, which shares 51.3% amino acid identity with VZV TK, is able to phosphorylate the BCNAs. The BCNAs proved to be a substrate for SVV TK, as ascertained by a time-dependent conversion of the BCNAs to their mono- and/or diphosphate derivatives in the reaction mixture. However, the structure–affinity relationship of the BCNAs for VZV TK and SVV TK was entirely different. Although the SVV TK was able to phosphorylate the BCNAs, no anti-SVV activity of any of the BCNAs could be detected in (BSC-l) cell cultures.

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Characterization of the DNA Polymerase Genes of Varicella-Zoster Virus (VZV) Resistant to Foscarnet (PFA) and the 2-Phosphonomethoxyethyl (PME) Derivatives of Adenine (Adefovir, PMEA) and 2,6-Diaminopurine (PMEDAP)

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The nucleotide changes of the DNA polymerase genes and the susceptibility profiles of several plaque-purified VZV mutants resistant to PFA and PME derivatives were determined. Six PFA^r clones revealed a single nucleotide change resulting in a K762E mutation, while one PFA^r clone showed in addition to the K762E mutation, a second mutation at position 874 (M8741). One $PMEDAP^r$ clone showed a single amino acid change in the DNA polymerase (D668Y) while two $PMEDAP^r$ clones contained a second mutation (M808V) in addition to the 668 mutation. One $PMEA^r$ clone revealed a unique mutation (L767S) in the DNA polymerase. All three groups of VZV mutants showed cross-resistance between the PME derivatives and PFA. Residues 662 and 668 are located in the conserved region II of the VZV DNA polymerase, while residues 767 and 808 are situated in the conserved region III. The residue 874 is located between the conserved regions I and VII. Residues K662, D668, L767, M808 and M874 in the VZV DNA polymerase correspond to the Q697, D703, L803, M884 and M909 residues, respectively, in the herpes simplex virus type I (HSV-l) DNA polymerase. The amino acid substitutions identified in this study conferring resistance to the PME derivatives and PFA are novel and have not been previously reported either in the VZV DNA polymerase or in the corresponding amino acid residues of the HSV-l DNA polymerase.

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Enhanced Metabolic Stability of Chlorinated Indole Nucleosides

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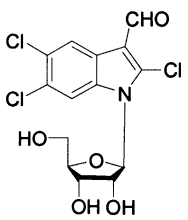
Many potent antiviral nucleosides undergo extensive first-pass metabolism in vivo. We have identified four enzymes responsible for cleaving the nucleoside glycosidic bond in vitro—3-methyladenine DNA glycosylase II (MPG), 8-oxoguanine DNA glycosylase (OGG1), purine nucleoside phosphorylase (PNP) and CYP3A4. These enzymes provide an in vitro screening tool for predicting metabolic stability of nucleoside analog drugs. This study utilized these nucleoside-metabolizing enzymes to investigate the glycosidic bond stability of several indole nucleosides with potent activity against HCMV. Initial cleavage rates of the parent indole, FTCRI, and three of its analogs were determined by MPG, OGG1, PNP and human liver microsomes, which contain CYP3A4, and the results were compared to those of a reference nucleoside, BDCRB. HPLC and subsequent data analysis yielded initial cleavage rates, reported as rate of aglycone appearance. Overall, the initial cleavage rate of each indole tested was significantly lower than that of BDCRB. Human liver microsomes did not significantly metabolize any of the indoles; the indoles were cleaved at 0–4% the rate of BDCRB. MPG, however, had mild affinity for one FTCRI analog, which was cleaved at approximately 17%, the rate of BDCRB, while FTCRI and the remaining analogs were cleaved between 1 and 7% the rate of BDCRB. OGG1 cleaved FTCRI at 20% the rate of BDCRB while the other analogs were cleaved at 0–8% the rate of BDCRB. PNP cleaved one FTCRI analog at 38% the rate of BDCRB, while FTCRI and the other analogs were cleaved at 0–9% the rate of BDCRB. These results indicate that indole nucleoside analogs exhibit far greater stability against metabolizing enzymes than the reference benzimidazole ribonucleoside, BDCRB.

Acknowledgment: This research was funded by NIH Grants GM37188 and 5T32-GM07767.



FTCRI

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Synthesis and Antiviral Activity of 9-[(3-hexadecyloxypropyl-phosphono)-1-propyloxymethyl]guanine

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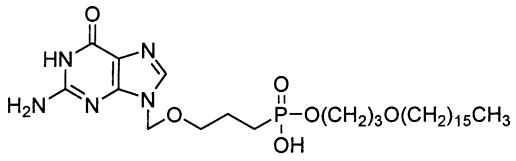
9-(3-Phosphonopropyloxymethyl]guanine (acyclovir phosphonate or PPMG) inhibits cytomegalovirus (CMV) replication in vitro. However, in vivo this compound may cause renal toxicity and may not be active orally. The antiviral activity of acyclic nucleoside phosphonates (e.g. cidofovir, HPMPA, PMEG) can be increased by esterification with long-chain alkoxyalkanols. We showed recently that the hexadecyloxypropyl ester of CDV (HDP-CDV) is orally bio-available, effective in animal models of viral disease, and decreases kidney exposure to drug compared to underivatized CDV. In this report we synthesized HDP-PPMG (**1**). PPMG was synthesized using the published methodology.[1] The phosphonic acid was esterified with 3-hexa-decyloxy-1-propanol in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC). PPMG and HDP-PPMG were tested for inhibitory activity against herpes simplex virus (HSV-1, MRC-5 cells by DNA reduction assay), HCMV (AD-169 in HFF cells by plaque reduction) and HIV-1 (MT2 cells by p24 reduction assay). Compound **1** was 580-fo1d more potent than PPMG in the HSV-1 assay (EC_{50} = 0.05 μM versus 28 μM for PPMG). HDP-PPMG was active against HCMV with an EC_{50} of 0.06 μM. The analog also inhibited HIV-1 replication (EC_{50} = 1.1 μM), but PPMG itself was inactive (EC_{50} > 100 μM). We conclude that PPMG esters such as HDP-PPMG are worthy of further evaluation as antiviral therapies.

Reference: [1] Reist et al., 1994. Nucleosides and Nucleotides 13, 539–550.



HDP-PPMG, **1**

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Direct Synthesis of Acyclic Nucleoside Phosphonate Alkoxyalkyl Monoesters

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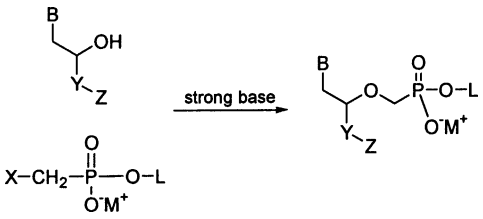
The oral efficacy of poorly absorbed antiviral phosphonates such as cidofovir, HPMPA and PMEG is improved when

they are esterified with long-chain alkoxyalkanols. For example, cidofovir hexadecyloxy-propyl ester (HDP-CDV) is active in several antiviral animal models when given orally, but unmodified CDV is not active orally. HDP-CDV is highly active against orthopoxviruses and is being developed as an oral therapy for smallpox. We initially prepared monoesters such as HDP-CDV from cyclic CDV using alkylation or condensation reactions followed by basic hydrolysis. In an attempt to improve efficiency before preparing larger quantities of HDP-CDV, we investigated more direct synthesis methods that incorporate the lipophilic portion of the molecule earlier in the process. The HDP ester of toluene-sulphonyloxymethylphosphonate (TsOMPA) can be obtained easily by esterification of the free phosphonic acid. HDP-TsOMPA reacts with protected 1-[(*S*)-2,3-dihydroxypropyl]cytosine in the presence of a strong base to provide protected HDP-CDV. Using this approach, maximum advantage can be taken of each protecting group, and simple aqueous/organic extraction procedures can be used to isolate the intermediates. This is a generally useful approach that can be used to prepare alkoxyalkyl monoesters of many acyclic nucleoside phosphonates.



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Synthesis and Antiviral Activity of 5-Substituted Hexadecyloxypropyl-1-[2-(phosphonomethoxy)-ethyl] cytosine (PMEC) Derivatives

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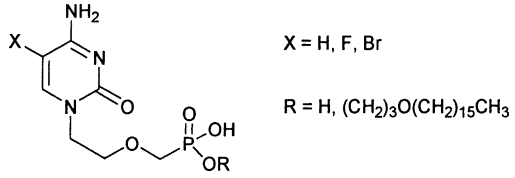
PMEC is related to the highly potent antiviral adefovir (9-[2-(phosphonomethoxy)ethyl]adenine, PMEA), which is active against retroviruses and several DNA viruses, and to cidofovir ((*S*)-l-[3-hydroxy-2-(phosphonomethoxy)propyl]-cytosine, HPMPC), which is useful against herpes and orthopox viruses. Despite its structural resemblance to these two acyclic nucleoside phosphonates, PMEC does not possess significant antiviral activity in vitro. However, PMEC's diphosphorylated adduct, PMECpp, strongly inhibits some viral DNA polymerases. One of the reasons PMEC lacks antiviral activity could be that PMEC is poorly taken up by infected cells. Previous work in our laboratory showed that long chain alkoxyalkyl esters of acyclic nucleoside phosphonates possess enhanced antiviral activity versus free phosphonates due to improved cellular penetration and metabolism. For this study we synthesized hexadecyloxypropyl (HDP) esters of PMEC, 5-fluoro-PMEC, and 5-bromo-PMEC, then tested the new compounds for antiviral activity in HSV-1, HCMV and HIV-1 infected cells.



HDP esters of 5-substituted PMEC showed substantially more inhibitory activity than the unmodified phosphonates. For example, HDP-5-fluoro-PMEC inhibited HSV-1 ($EC_{50} = 0.01\,\mu M$) but 5-fluoro-PMEC was much less active ($EC_{50} => 50\,\mu M$). Conversion to lipophilic alkoxyalkyl esters appears to be a general method that can increase the antiviral effects of highly polar (dianionic) phosphonates.

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New Class of Fluoro Substituted Methylenecyclopropane Analogues of Nucleosides as Antiviral Agents: Synthesis and Antiviral Activity

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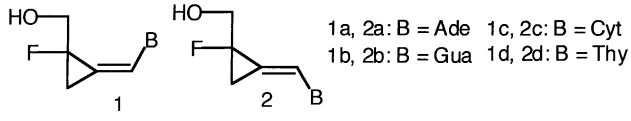
The title compounds **1a**–**1d** and **2a**–**2d** were synthesized and their antiviral activity against HCMV, HSV-1, HSV-2, EBV, VZV HIV-1 and HBV was investigated. Adenine Z-analogue **la** was highly active against HCMV (Towne and AD169)/HFF with EC_{50}/CC_{50} 3.6/>100 and 6.0/>425 μM, respectively, but it was less potent against MCMV/MEF (69.3/>425 μM). In the *E*-series, adenine derivative **2a** was the most effective against HCMV/HFF [EC_{50}/CC_{50} 20/>100 (Towne) and >16.7/>417 μM (AD169)]. Against EBV/Daudi *Z*-isomers **1a**, **1b** and **1c** were potent inhibitors with $EC_{50} < 0.38$–4.8 μM and *E*-isomers **2a**, **2b** and



2c were also effective (EC_{50} 0.32–2.3 μM). No cytotoxicity was observed in both series. In contrast, only analogues **1d** and **2a** exhibit significant potency against EBV/H-1 (EC_{50}/CC_{50}(CEM) 2.5 and 3.6/>100 μM, respectively). Pyrimidine analogues **1c** and **1d** were effective against VZV/HFF with EC_{50}/CC_{50} 0.62/>400 μM. Compound **1d** inhibited also replication of HSV-1 in BSC-1 (ELISA) with EC_{50} 2.5 μM. Adenine *E*-analogue **2a** was the only effective anti-HBV agent in 2.2.15 cells (EC_{50} 15 μM, CC_{50} in CEM > 100 μM). Surprisingly, the *E*-isomer **2a** was more potent against HIV-1/MT-4 than *Z*-isomer **1a** (EC_{50}/CC_{50}) 5.0/>100 versus 17/>100 μM). Adenine analogues **1a** and **2a** are substrates for adenosine deaminase. Comparison of antiviral activity of fluorinated and non-fluorinated analogues **1** and **2** (F = H) revealed significant differences which will be discussed.

Acknowledgment: This research is supported by NIH grants RO1-CA32779, NO1-AI85347, RO1-44358 and PO1-46390.

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In Vitro Activity of Second Generation Methylenecyclopropane Analogs of Nucleosides Against Herpesvirus Replication

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We have reported previously that purine 2-(hydroxymethyl) methylenecyclopropane analogs have demonstrated good activity against the herpesviruses. A second generation of methylenecyclopropane analogs, the 2,2-bis-hydroxymethyl derivatives was synthesized using an alkylation-elimination approach reported previously for 2-(hydroxymethyl) methylenecyclopropanes. Twenty-six compounds were tested for activity in vitro against human and murine cytomegalovirus (HCMV, MCMV), herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), and Epstein-Barr virus (EBV). Selected analogs were also evaluated for activity against human herpesvirus type 6 (HHV-6) and human herpesvirus type 8 (HHV-8). Six of the compounds were active against HCMV by cytopathic effect (CPE) or plaque reduction (PR) assays (0.5–22 μM). Four of the six compounds were also active against MCMV by PR (0.3–9.7 μM). Eight of the compounds were active against VZV by CPE (0.6–l4 μM) and nine of the compounds were active against EBV by ELISA (<0.3–4.8 μM). Four compounds were active against HHV-6 (0.7–28 μM) and two were active against HHV-8 (6.5–l6 μM). There was no activity against HSV-1 or HSV-2. Toxicity was evaluated in adherent and non-adherent cell lines and minimal toxicity was observed. One of these analogs, ZSM-I-62 which is (*Z*)-9-{[2,2-bis-(hydroxymethyl)cyclopropylidene]methyl} guanine had particularly good activity against the β herpesviruses tested with EC_{50} values of 0.5–8 μM. This compound has also been evaluated in animal studies and appears to be a good candidate for HCMV and HHV-6 infections. These results indicate that some members of this series of second generation methylenecyclopropane analogs were highly active against HCMV, MCMV, HHV-6A, and HHV-6B, were less active against EBV and HHV-8 and were inactive against HSV-l and HSV-2. The analog, ZSM-I-62 was particularly active against the β herpesviruses and should be evaluated further for use in these infections in humans.

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Use of Clustering Algorithms for Analysis of Chemotypic Data to Help Elucidate the Mechanism of Action of New Antiviral Agents

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Antiviral drugs can be classified by their chemical structure, spectrum of activity and mechanism of action. It is also possible to classify compounds by the characteristic biological changes that they induce in infected cells. A group of 20 diverse antiviral drugs were evaluated in human cytomegalovirus-infected human foreskin fibroblast cells and the effect that each of these molecules had on viral replication, DNA synthesis and expression of representative viral proteins was characterized. The susceptibility of recombinant viruses with specific deletions in UL97, UL27, and UL114 were also measured to help identify viral gene products involved in the activation of these compounds. The resulting data matrix was subjected to a clustering analysis in order to group compounds that elicited similar biological effects in infected cells. This analysis clustered nucleoside and non-nucleoside inhibitors of DNA polymerase together and distinguished compounds that required activation by UL97 from other inhibitors such as cidofovir that have no such requirement. Inactive compounds and compounds that inhibited late stages in viral replication also formed separate and distinct clusters. This approach proved to be useful in helping to elucidate the mechanism of action of new compounds by relating their effects to those induced by other compounds with well-defined mechanisms of action. This type of analysis should also be able to relate the phenotypes associated with specific viral genes with the characteristic effects, or chemotypes of antiviral drugs to improve our understanding of their modes of action and their functions in viral replication.

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Cellular Uptake of Cidofovir and Various Alkoxyalkyl Esters of Cidofovir in MRC-5 Cells: Comparison with Antiviral Activity

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Alkoxyalkyl esters of cidofovir (CDV) such as hexadecyloxypropyl-CDV (HDP-CDV) have shown a substantial increase in antiviral activity compared to unmodified CDV against such viruses as HSV-1, HCMV and orthopoxviruses. In contrast to CDV, they are orally bioavailable and have been reported to be orally active in lethal cowpox virus infection in mice. We previously studied the cellular uptake and metabolism of CDV, HDP-CDV and their cyclic counterparts and showed that levels of CDV diphosphate, the active metabolite, were >100 times greater with HDP-CDV than with CDV. We now have examined the cellular uptake of octa-decyloxyethyl- (ODE-CDV), oleyloxypropyl- (OLP-CDV) and octadecyl-2-benzyl-glycerol- (ODBG-CDV) esters of ^{14}C-labeled CDV and compared uptake results with the antiviral activity of the compounds. Briefly, 3.0 μM ^{14}C-labeled drug was added to subconfluent MRC-5 human lung fibroblast cells and incubated at 37 °C for 0.5–24 h. The drugs were removed and the monolayer washed. The cell monolayer was lysed with sodium hydroxide and counted. We also exposed MRC-5 cells to varying concentrations of drug, 0.3–3.0 μM and determined cell uptake at 4 h. In the time course experiment, the compound with the highest antiviral activity, ODE-CDV, had the highest cellular uptake, 73 times greater than CDV at 24 h. The other CDV esters also had greatly increased cell uptake compared with CDV. The dependence of cell uptake on drug concentration showed the same order of activity as the 24 h uptake studies with ODE-CDV > HDP-CDV and ODBG-CDV > OLP-CDV ≫ CDV. The antiviral activity was not strictly a function of cell uptake. To correlate antiviral activity with cellular uptake and metabolism, it will be necessary to determine the comparative cellular levels of the active metabolite, CDV-diphosphate. In summary, all of the highly active esters of CDV studied thus far, exhibit greatly increased cellular uptake versus CDV.

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Mucosal Immunization with a Replication-Deficient Adenovirus Vector Expressing Glycoprotein H of Murine Cytomegalovirus Induces Mucosal and Systemic Immunity

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We have examined the ability of mucosal immunization with a replication-deficient adenoviral vector expressing glycoprotein H of murine cytomegalovirus (MCMV) to alter host immunity. A vaccine vector was constructed by inserting the complete ORF of MCMV gH under control of the human CMV IE-1 promoter into the E-1 region of a replication-deficient adenovirus 5. This vector, designated Ad-gH, was then propagated in QB1-293 cells to generate vaccine pools. In vitro infection of QB1-293 cells and mouse embryo cells with Ad-gH resulted in expression of MCMV gH detected by both Western blot and IFA. Immunization of BALB/c mice with Ad-gH (1×10^7 PFU) given by the intranasal route induced a humoral response with antibody detected in serum of 100% of vaccines. Antibody to MCMV gH was also detected in the bronchoalveolar lavage, fecal suspensions and vaginal washings. The viral titer of lung and salivary gland of vaccinees 10 days after intranasal challenge with MCMV (1×10^5 or 1×10^3 PFU) were significantly reduced compared to controls, but virus infection was not prevented. Re-exposure of vaccinees to Ad-gH 30 days after primary immunization induced a remarkable boost serum and mucosal antibody responses. When rechallenged with MCMV intranasally, these mice had further reduction of MCMV titers in the lung and salivary glands. The suppression of MCMV titers in the lung and salivary gland was augmented. Such a strategy may be important in reducing horizontal transmission of CMV infections across mucosal surfaces and in altering host immunity to CMV.

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Search and Assessment of Novel Substances Active Against Epstein-Barr Virus

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The search for the novel antiviral substances active against Epstein-Barr virus (EBV) is a topical problem since the persistent EBV infection alters immune status promoting the development of adenocarcinomas and lymphoproliferative diseases. The aim of the study was to assay the

anti-EBV activity of several substances prepared from the raw material of the plant origin, namely Altabor and Proteflasid. Altabor (Borschahovskyi Chemical and Pharmaceutical Plant, Kyiv) based on the polyphenols isolated from infructescences of the alder comprises monomers and olygomers of ellongotannins (no less than 60%), melanoid polymer (about 10%), and phenolic acids (ellagic, gallic, valonic acids as well as mono- and polysaccharides). Proteflasid (Ecopharm Research and Production Company, Kyiv) represents the quercetin-containing herbal extract of wild grasses *Deschampsia caespitosa* L. and *Calamagrostis epigeios* L. Anti-EBV activity of the substances was assessed in EBV-infected lymphoblastoid Raji cells. The substances were assayed within broad concentration ranges. The maximally tolerable concentrations for the cell line being assayed amounted to 1000 μg/ml for Altabor and 150 μg/ml for Proteflasid. The inhibition of EBV reproduction was assessed by PCR technique estimating the number of EBV DNA genomic equivalents. Minimal effective concentrations amounted to 2 μg/ml for Altabor and 0.1 μg/ml for Proteflasid. Therefore, the chemotherapeutic indices for Altabor and Proteflasid were estimated as 500 and 1500, respectively. To summarize, the substances under study possess the significant anti-EBV activity and may be the advantageous for the therapy of EBV-associated diseases.

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Activity of Novel Acyclic Pyrimidine Nucleoside Phosphonates Against Poxviruses

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We have recently reported the discovery of a novel class of antivirally active acyclic nucleoside phosphonates (ANPs) in which the base consists of a pyrimidine containing an amino group at C-2 (DAPY) and a 2 (phosphonomethoxy)ethoxy (PMEO) or a (*S*)-[3-hydroxy-2-(phosphonomethoxy)propoxy] (HPMPO) group at C-6. We have now evaluated the activity of these new ANPs against various poxviruses, including two strains of vaccinia virus (VV, Lederle and Lister), cowpox virus (Brighton strain) and several ORF virus (ORFV) strains in primary human keratinocytes (for VV and cowpox virus) and primary lamb keratinocytes (for ORFV). HPMPO-DAPY was able to inhibit the replication of the different poxviruses with 50% inhibitory concentration (IC_{50}) values in the range of 1.0–7.5 μg/ml, compared to 0.5–6 μg/ml for cidofovir [(*S*)-1-[3-hydroxy-2-(phosphonomethoxy)-propyl]cytosine, HPMPC], and 0.6–2 μg/ml for HPMPDAP [(*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]-2,6-diaminopurine. PMEO-DAPY did not show activity against any of the ORFV strains tested, while mean IC_{50} values for VV Lister strain and Lederle strain and cowpox virus were $\geq$29, 12.5 and 30 μg/ml, respectively. On the other hand, 2-(phosphonomethoxy)ethyl (PME) derivatives of adenine (adefovir, PMEA) and 2,6-diaminopurine (PMBDAP) did not show activity against any of the poxviruses tested. We are currently evaluating the effects of HPMPO-DAPY, in comparison to HPMPC and HPMPDAP, against the different poxviruses in organotypic epithelial raft cultures.

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In Vitro Activity of Alkyl Esters of Cidofovir and Cyclic Cidofovir Against Orthopoxvirus Replication: Comparison of Activity with Alkoxyalkyl Esters

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We have reported previously that a new series of alkoxyalkyl esters of cidofovir (CDV) and cyclic cidofovir (cCDV) were more efficacious than the parent compounds against vaccinia and cowpox viruses in vitro, with EC_{50} values ranging from 0.06 to 16 μM for the analogs compared to values of 31–45 μM for CDV and cCDV. Subsequent synthesis and evaluation of additional analogs and of structure–activity relationships indicated that the activity of these compounds was strongly dependent on chain length and the presence or absence of a double bond in the alkoxyalkyl chains linked to the phosphonates moiety of CDV. To assess the need for the oxygen heteroatom, we synthesized a series of alkyl esters of CDV for comparison. Alkoxyalkyl and alkyl esters of CDV having chains shorter than 16 atoms were generally less active. Derivatives with no linker and with the oxypropyl linker had optimal chain lengths of 18–20 atoms beyond the phosphonates, with activity declining slightly to 24 atoms. Cyclic CDV analogs had a sharp chain length optimum for antiviral activity at 20 atoms. The presence of a 9,10 *cis* double bond in the 18 carbon alkyl chain (oleyl-) increased the activity about three-fold relative to the saturated alkyl chain (octadecyl). The oxyethyl analogs of CDV (ODE- and OLE-CDV) were generally more active than their oxypropyl counterparts (ODP- and OLP-CDV) even though they differ in overall number of atoms by only one methylene. OLE-CDV was the most active and selective derivative, with an EC_{50} of 0.06 μM and a SI of 933 against vaccinia virus and an EC_{50} of 0.07 μM and a SI of 800 versus cowpox virus. In comparison, the SI of OLE-CDV was about 100-fold greater than CDV.

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HDP-(*S*)HPMPA: Oral Pharmacokinetics and Antiviral Activity Against Orthopoxvirus and Murine CMV Infections in Mice

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We reported previously that HDP-(*S*)HPMPA is highly active in vitro against orthopoxviruses and CMV. In this study, we examined the oral bioavailability of ^{14}C-labeled HDP-(*S*)HPMPA in mice. To evaluate pharmacokinetics, a single oral or intraperitoneal (i.p.) dose of 10 mg/kg of ^{14}C-labeled HDP-(*S*)HPMPA was administered to mice. Blood, spleen, kidney, lung, liver, heart, and brain were obtained at 1, 3, 6, 12, and 24 h and aliquots were counted and drug content was determined. Peak plasma levels of ^{14}C-HDP-(*S*)HPMPA after oral administration were 1.1 μM at 1 h declining slowly to 0.028 μM at 24 h. Peak plasma levels of HDP-(*S*)HPMPA given i.p were 2.2 μM declining to 0.085 μM at 24 h. Based on the respective plasma areas under curve from 0 to 24 h, the relative oral bioavailability of HDP-(*S*)HPMPA was calculated to be 72%. Groups of 15 female BALB/c mice, 3 weeks of age, were infected intranasally with cowpox virus (Brighton strain), vaccinia virus (WR strain) or intraperitoneally with murine cytomegalovirus (Smith strain). After 24, 48 or 72 h, HDP-(*S*)HPMPA, 3, 10 or 30 mg/kg, was administered orally daily for five days. Drug treated, uninfected controls showed no toxic effects but untreated, infected animals exhibited 100% mortality. With cowpox, 30 mg/kg reduced mortality by 87% after treatment delays of 24–72 h. Ten milligram per kilogram showed protection after 24 or 48 h after infection but 3 mg/kg was ineffective. Generally similar results were noted with MCMV after treatment delays of 24 or 48 h. Preliminary experiments with vaccinia virus also showed protective activity after treatment delays of 24 h.

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Effect of (*S*)-HPMPDAP and (*S*)-HPMPO-DAPym on Vaccinia Virus Infections in Mice

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There is a need to develop strategies to treat smallpox infections should they reappear. It is also important to have an effective drug at hand for the treatment of monkeypox disease in humans and to treat complication of vaccination against smallpox. We show here that (*S*)-9-[3-hydroxy-2(phosponomethoxy)propyl]-2,6-diaminopurine [(*S*)-HPMPDAP] and 2,4-diamino-6-[3-hydroxy-2-phosphonomethoxy)-propyloxy]pyrimidine [(*S*)-HPMPO-DAPym] are potent inhibitors of the replication of the vaccinia virus (VV) in cell culture. This was corroborated by a potent and dose-dependent inhibition of vaccinia DNA synthesis in human embryonic lung cells, as determined by real time Q-PCR [EC_{50} for (*S*)-HPMPDAP <0.006 μg/ml, for (*S*)-HPMPO-DAPym 0.064 μg/ml and for (*S*)-HPMPC 0.025 μg/ml]. The in vivo protective effect of (*S*)-HPMPDAP, (*S*)-HPMPO-DAPym and (*S*)-HPMPC was next assessed in the VV tail lesion model in immunocompetent mice. Doses of 25, 10, 2.5 and 1.0 mg/kg per day [given for five consecutive days (starting at 2 h post infection)] of (*S*)-HPMPO-DAPym and (*S*)-HPMPDAP proved equipotent to (*S*)-HPMPC in reducing the number of tail lesions. The effect of the compounds was next determined in a lethal model for disseminated VV infection in intracutaneously infected athymic nude (*nu*/*nu*) mice. *Nu*/*nu* mice were infected intracutaneously at the lumbosacral area with VV. The infection was allowed to proceed for 14 days after which systemic treatment with either compound was initiated (at 100 or 50 mg/kg per day according to different treatment schedules). (*S*)-HPMPO-DAPym, but not (*S*)-HPMPDAP, was able, akin to (*S*)-HPMPC, to halt progression of the infection and to cause complete healing of the lesions.

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A Non-Nucleoside Related to Isoniazid Exhibits Potent Anti-Orthopoxvirus Activity in Cell Culture but Not in Mice

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1-Adamantoyl-2-isonicotinoylhydrazine chlorohydrate (D-95) was evaluated for antiviral activity against several DNA and RNA viruses in cell culture. It was found to be active against orthopoxviruses but not other viruses. The 50% inhibitory concentrations of D-95 against cowpox (Brighton strain) and vaccinia (WR strain) viruses in Vero cell plaque assays were 1.6 and 0.4 μM, respectively. In contrast, cidofovir (a positive control that is a drug candidate for treatment of smallpox in humans) was inhibitory to these viruses at 50–60 μM. Isoniazid did not inhibit these two viruses. D-95 was equally active against wild-type and cidofovir-resistant vaccinia viruses. In virus yield reduction assays, D-95 inhibited vaccinia virus by 90% at 1 μM. Cidofovir was similarly active at 35 μM. D-95 was 50% inhibitory to actively replicating cells at 500–1000 μM, giving it a high selectivity index. Mice infected intranasally with vaccinia virus were

treated twice daily with D-95 at 100, 50, and 25 mg/kg per day for 5 days starting 24 h after virus exposure. Such treatments did not prevent death, and the time to death was not increased compared to placebo-treated animals. Lung and snout virus titers (determined on day 5 of the infection) were not reduced by D-95 treatment. A second experiment was conducted evaluating D-95 at 200 mg/kg per day, administered either twice daily or three times daily. These treatments were unable to change the outcome of the lethal vaccinia virus infection. The data suggest poor metabolic properties of D-95 in animals, thus more suitable derivatives of this compound are being sought.

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Isolation of Vaccinia Virus (VV) Mutants Resistant to Different Acyclic Nucleoside Phosphonate Analogues (ANPs)

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Acyclic nucleoside phosphonates (ANPs), particularly cidofovir [1-(*S*)-(3-hydroxy-2-phosphonomethoxypropyl)cytosine, HPMPC], are good candidates for the treatment and prophylaxis of poxvirus infections, based on the efficiency of cidofovir against vaccinia, cowpox and monkeypox viruses both in vitro and in different animal models. We have now isolated drug resistant mutants of vaccinia virus (VV) under the selective pressure of various ANPs, including cidofovir, cyclic HPMPC (cHPMPC), the HPMP derivatives of adenine (HPMPA) and 2,6-diaminopurine (HPMPDAP) and cyclic HPMPA (cHPMPA). After 30–40 passages in the presence of increasing concentrations of the compounds, the different mutants were tested for their sensitivity to the different ANPs analogues in a CPE reduction assay in human embryonic lung (HEL) fibroblasts. The HPMPC^r, cHPMPC^r, HPMPA^r, cHPMPA^r and HPMPDAP^r mutants showed resistance not only to the HPMP derivatives but also to (*S*)-2,4-diamino-6-[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (HPMPO-DAPY), a new class of ANPs with activity against poxviruses. Interestingly, the different VV mutants did not show a significant increase in $1C_{50}$ values for 3-deaza-HPMPA. As we have previously described for herpesviruses, no cross-resistance with the PME [2-(phosphonomethoxy)ethyl] derivatives was observed. Various HPMPC^r and HPMPDAP^r plaque-purified clones have been isolated and characterized phenotypically. The nucleotide sequence of the DNA polymerase gene of these clones is currently being assessed to identify the genotypic change(s) responsible for the resistant phenotype.

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Antiviral Ribonucleosides: Inhibitors of Inosine Monophosphate Dehydrogenase

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Inosine monophosphate dehydrogenase (IMPDH, EC 1.1.1.205) catalyzes the oxidative conversion of IMP to XMP, utilizing the coenzyme, NAD+, as the hydride acceptor. It has been considered as an important target enzyme for the development of chemotherapeutic agents, including antiviral agents. The molecular design of inhibitors of IMPDH in this investigation was based on the mechanism of substrate action of IMPDH which involves covalent interaction of the enzyme–coenzyme complex at the 2-position of IMP. Thus, for the target compounds in this study, emphasis was placed on specific modifications at this position that facilitate this covalent interaction. Synthesis of inhibitors utilized chemoenzymatic methods for the functionalization of the 2-position of the hypoxanthine ring and subsequent enzymatic phosphorylation of the modified ribonucleosides. Highly purified ribonucleotides were produced by HPLC separation. Inhibition studies with IMPDH were carried out spectrophotometrically by monitoring the formation of NADH. Antiviral data were obtained through collaborative studies. The chemoenzymatic synthesis, enzyme kinetic studies and antiviral data will be analyzed and explained.

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Combinatorial Antibodies Against Orthopoxviruses

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Variola virus, a member of the *Orthopoxvirus* genus, is a highly pathogenic agent specific for human beings. All the variola virus strains can be distinguished on the basis of disease they caused and the mortality rate: *variola major*, which caused the classic disease; and *variola minor* (its South American strains are known as *alastrim*), which caused disease with a lower fatality rate and was endemic along with *variola major* strains in America and Africa. The mechanisms underlying variola's pathogenicity and strain specificity are not yet well-understood. Three collections of human scFv phage antibodies against live *variola virus Ind-3a*, (major strain), *variola virus Butler*, (alastrin strain) and *vaccinia virus Elstree* were developed form a combinatorial phage library of human single-chain antibody fragments (Medical Research Council Centre, Cambridge, England). Selected phage antibodies were used for ELISA with different strains of *variola virus* to find strain-

specific antibodies, and several of them were shown to bind the strains from *alastrim* group more effective then other variola strains. Amino acid motif was found in Vh CDR3 of 'selective'antibodies after sequencing of scFv genes. Target proteins of the selective antibodies were identified using immunoblot analysis.

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vvG1L: An Antiviral Drug Target

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Variola virus (smallpox) is a Category A pathogen considered to be one of the most significant threats for use as a bioterrorism agent. Due to complications from vaccination, mass immunization of the populace is contra-indicated. Our current research seeks to develop effective anti-orthopox drug(s). Using vaccinia virus (VV) as a model system, the goal of our previous research was to determine if the 17L cysteine proteinase or the G1L metalloproteinase encoded by VV is the poxvirus core protein proteinase (vCPP), and to use this information to develop vCPP inhibitors as candidate antiviral drugs. We have recently demonstrated that the 17L cysteine proteinase is the vCPP [J. Virol. 76 (2002) 8973] and are proceeding with drug development efforts on this target. But what about G1L? We believe that the vvG1L metalloproteinase represents a unique and distinct orthopoxvirus antiviral target. The purpose of the experiments currently underway are to: (1) Produce a G1L conditional-lethal mutant to assess the phenotype of the null mutant; (2) Elucidate the biological role of GIL during viral replication and/or assembly; and (3) Demonstrate and characterize the enzymatic activity of the G1L gene product. Successful completion of these experiments should allow the development of G1L metalloproteinase inhibitors as antiviral drugs to be initiated. There are several important reasons to exploit the G1L target in addition to 17L: Not all enzymes are equally "drug-able" and G1L inhibitors may have superior activity/specificity profiles; when exposed to selective pressure, viruses rapidly acquire resistance so having multiple antiviral drugs available is essential; and using a cocktail approach with multiple inhibitors may be more effective than using a single drug.

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vvI7L: An Antiviral Drug Target

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Development of an effective antiviral drug requires the identification of a specific interaction or activity whose disruption will be lethal to the virus and relatively benign to the host. Since viruses, such as orthopoxviruses, are obligate intracellular parasites which utilize many of the host cell enzymes and metabolic pathways during their replication, this task has proved quite difficult and this fact is chiefly responsible for the absence of successful antiviral drugs for use against smallpox. Fortunately, the realization that viruses use proteolysis catalyzed by viral-encoded proteinases as a key step in their developmental cycle, has opened up a new class of targets for antiviral drug development. In recent work, we have identified the poxvirus gene that encodes the viral core protein proteinase (vCPP). This gene (17L) is highly conserved in all pathogenic poxviruses and is predicted to encode a cysteine proteinase. We have taken advantage of this attractive target to develop an effective drug that blocks orthopoxvirus replication based on specific mechanistic inhibition of vCPP. Based on homology-based computational bioinformatics, a structural model of the 17L proteinase was developed. A unique chemical library of ~450,000 compounds was computationally queried to identify potential active site inhibitors. The resulting biased subset of compounds was assayed for both toxicity and the ability to inhibit the growth of vaccinia virus in tissue culture cells. Phenotypic analyses of virus-infected cells were conducted in the presence of active compounds to verify that the correct biochemical step (core protein processing) was being inhibited. By generation of a drug resistant viral mutant by many passages in the presence of the drug, we were able to verify that the induced mutation maps to the 17L gene. We have selected a small number of compounds to evaluate in greater detail, the best of which have IC_{50} of ~2 μM and therapeutic indices >150.

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Activity of Ether Lipid Esters of Acyclic Nucleoside Phosphonates Against Adenovirus Replication In Vitro

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There is a worldwide incidence of adenovirus (AdV) infections, with respiratory illnesses, gastroenteritis, and ocular infections being the most common and 49 distinct serotypes of AdV are associated with human infection. Most AdV infections are usually mild or asymptomatic but they can cause significant morbidity and mortality in the immunocompromised host. There is currently no approved treatment for these infections. The acyclic nucleotide phosphonate, Cidofovir (CDV) and its closely related analog, (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ((*S*)-HPMPA), have demonstrated activity against many

AdV serotypes. However, the activities of these compounds are serotype-dependent and therefore may not be effective against all serotypes. The development of alkoxyl esters of CDV and (*S*)-HPMPA may provide potential orally active antiviral agents for AdV infections. CDV, the cyclic ester of CDV (cCDV) and the alkoxyalkyl esters of CDV (HDP-, ODE-, OLP-, OLE-) and (*S*)-HPMPA (HDP-, ODE-) exhibited antiviral activity against the majority of AdV serotypes tested. CDV had EC_{50} values from 1.3 to 2.0 μM and cCDV from 1.8 to 3.5 μM for strains 3, 5, 7, and 8. AdV 31 was not susceptible to either compound. The analogs of CDV had multi-fold increased EC_{50} values over the parent compound including activity to AdV 31 which was resistant to CDV. The most active compounds were ODE-CDV (EC_{50} < 0.006–0.09 μM) and HDP-CDV (EC_{50} < 0.0009–0.28 μM). (*S*)-HPMPA had EC_{50} values ranging from 0.5 to 2.5 μM. HDP-IIPMPA had similar EC_{50} values of 0.19–26 μM with AdV 31 again not being susceptible. In contrast, ODE-HPMPA had EC_{50} values of 0.002–0.07 μM, including AdV 31. The most active compound against each serotype was ODE-CDV, followed by HDP-CDV and OLE-CDV. The toxicity of all the analogs was greater than the parent compounds, but at the EC_{50} level they were non-toxic. These studies indicate that sever analogs of CDV and (*S*)-HPMPA have potent activity against many AdV serotypes and should be evaluated further for their potential use in human AdV infection.

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Activity of Different Classes of Nucleoside and Nucleotide Analogues Against Adenovirus in Cell Culture

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Although adenovirus infections are clearly recognized as important pathogens in immunocompromised patients (such as transplant recipients), therapeutic options are currently limited to the acyclic nucleoside phosphonate (ANP) analogue cidofovir. We initiated an antiviral study to compare the activity of different classes of compounds against human adenovirus in cell culture, based on evaluation of the cytopathic effect, MTS-based cell viability staining, or quantitation of viral DNA by QT-PCR analysis. The most active and selective compounds were: the ANPs cidofovir and the novel derivative (*S*)-2,4-diamino-6-[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine, and the 2′,3′ dideoxynucleoside analogues alovudine (FddT) and zalcitabine (ddC). We next determined the anti-adenovirus activity of acyclic nucleoside analogues (i.e. ganciclovir) in human osteosarcoma cells deficient for cytosolic thymidine kinase (TK), and in a cell line derived thereof expressing HSV-l TK. Based on Western blot analysis of viral proteins at 48 h after infection, ganciclovir was found to completely inhibit adenovirus replication at a concentration of 100 and 0.008 μM, in the parent osteosarcoma cells and the HSV-l TK transfectant, respectively. Thus, the poor efficacy of ganciclovir against adenovirus in cell culture or in patients cannot be explained by insufficient inhibition at the level of the adenovirus DNA polymerase, but rather by the absence of a virally encoded thymidine kinase. Collectively, our antiviral data show that the adenovirus DNA polymerase has a relatively broad substrate specificity, enabling its further exploitation as an antiviral target in anti-adenovirus drug development programs.

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Antiviral Activity of New Triazine Nucleoside Analogues

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Adenovirus (Ad) may induce several acute clinical syndromes such as respiratory, gastrointestinal and ocular diseases. They can cause life-threatening infection in the immunocompromised patient. Now is the only effective antiadenoviral agent, which licensed for human use. This is topical gel of Cidofovir (Forvade) used in the treatment of adenovirus keratoconjunctivitis. Nevertheless, drugs for treating other forms of adenoviral infection are not yet available. Earlier antiviral activity of 6-azacytidine and some its derivatives (N_4-substituted derivatives and derivatives modified on sugar part) had been revealed. In the present study the new triazine nucleoside analogues were synthesized by modified Vorbruggen method and were tested for cytotoxic and antiviral activity against AdS in HeLa cell culture. Screening of these compounds was conducted with a help of specific quantitative test developed by us, which was based on a detection of adenovirus-infected cells according to presence of viral intranuclear DNA-containing inclusions in them. Minimum inhibitory concentration (MIC) of the compound was considered that, with which the compound had suppressed amount of infected cells on 50%. Our results show that inhibitory effect of 2-thio-6-azacytidine is more strong and specific then other synthesized compounds. MIC of 2-thio-6-azacytidine is 2 μg/ml and its therapeutic index −30. These data suggest that 2-thio-6-azacytidine is a potential inhibitor of Ad reproduction.

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A Colorimetric Cell Culture Assay for the Identification of SARS Coronavirus Inhibitors

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Severe acute respiratory syndrome (SARS) has recently emerged as a new severe human disease, resulting globally in 774 deaths from 8098 reported probable cases. A novel member of the *Coronaviridae* family has been identified as the causative agent of this pulmonary disease. Although the initial global outbreak of SARS appears to have been successfully contained, SARS will remain a serious concern while there continues to be no suitable vaccine or effective drug treatment. A colorimetric assay based on the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) into a water soluble formazan product which can be directly quantified using a microtiter ELISA reader, has been developed for SARS coronavirus strain Frankfurt 1 drugsusceptibility testing. Optimal conditions were determined and the standard routine assay was calibrated with a viral input of 100 $CCID_{50}$ and a density of 10,000 cells per well in a 96-well microtiter plate for an incubation period of 3 days. Interferon β was used as a positive control to validate the assay. The effective IC_{50} concentration value obtained with interferon β in the present assay was in agreement with interferon β activity results published by others. This method presents the advantage of being rapid, reliable, reproducible, and convenient for high throughput screening capacity in a stringent P3 biosafety environment.

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Antiviral Activity of Glycyrrhizic Acid (GL) Derivatives Against SARS-Coronavirus (SARS-CoV) and Human Cytomegalovirus (HCMV)

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Glycyrrhizic Acid (GL) is the major bioactive triterpene glycoside of licorice root (Glycyrrhiza Radix). The antiviral activity of GL against a broad spectrum of viruses, among others HIV, HSV1, Influenza Virus, SARS-CoV, HBV and HCV has been reported.

In the effort to discover GL analogous with strong enhanced antiviral activity, we tested a number of new synthesized GL derivatives against HCMV and SARS-CoV. These compounds were received by introduction of different functional groups in the Carboxyl and Hydroxyl groups as well as transformations of the carbohydrate part of the molecule.

Our results show that the GL-amides BL43 and BL49, the reduced GL-trimethyl ester BL 44, and the Glycopeptide L-Cys-GL present a more than 10-fold increased antiviral activity against SARS-CoV compared to Glycyrrhizin, while the GL-amides BL26, and BL43 presented antiretroviral activity against HCMV.

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Antiviral and Virucidal Activities of Oreganol P73-based Spice Extracts Against Human Coronavirus In Vitro

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Human Coronavirus (HCoV) infection is very common, disseminated by air and occurs worldwide. Recently, a previously unknown HCoV has been implicated as a causative agent of severe acute respiratory syndrome (SARS). Finding a successful antiviral drug for SARS-associated HCoV is particularly challenging. The virucidal and antiviral activities of two Oreganol P73-based spice extracts were evaluated during in vitro HCoV (ATCC VR-740) infection. To determine, the virucidal potentials of non-cytotoxic dilutions of Oreganol P73 Extra Strength Formula (0.1%) and Oregacyn (0.01%), the virus was exposed to each drug dilution, and samples were collected at various times post-exposure prior to assay on the host cells. The antiviral ability of both Oreganol P73 Extra Strength Formula and Oregacyn was determined by maintaining the non-cytotoxic concentration of both drugs throughout the viral–host interaction period. The cell culture plates were examined microscopically for the presence of HCoV-induced cytopathic effects produced by viral infection. The virucidal part of the study indicated that both Oreganol P73 Extra Strength Formula and Oregacyn at final concentrations of 0.1 and 0.01%, respectively, proved to be coronavirucidal in direct proportion to exposure time ranging from 2 to 20 min at ambient temperature. In contrast, the antiviral studies revealed both Oreganol P73 Extra Strength Formula and Oregacyn completely inhibited HCoV infection in vitro. These data indicate the potential value of these Oreganol P73-based spice extracts as anti-HCoV compounds and merit further investigation against other mammalian viruses including HIV, HBV, HCV, influenza-, parainfluenza-, respiratory syncytial virus, herpes-, Hanta- and West Nile viruses.

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Mannose-Specific Plant Lectins are Potent Inhibitors of Coronavirus Infection Including the Virus Causing SARS

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Plant lectins are known to inhibit the infection of viruses that contain a glycosylated envelope such as human immunodeficiency virus, cytomegalovirus and influenza virus. A broad variety of plant lectins with specificity for α-1,3 and/or α-1,6 oligomannosides, *N*-acetylglucosamine and *N*-acetylgalactosamine have now been evaluated for their inhibitory activity against feline coronavirus (FIPV-1146)- and human (SARS) coronavirus (Frankfurt strain-I) (SARS CoV)-induced cytopathicity in Crandell feline kidney (Crfk) and Vero cell cultures, respectively. The majority of the plant lectins was not markedly effective against both viruses at concentrations as high as 100 μg/ml. However, the *N*-acetylglucosamine specific Urtica dioica agglutinin (UDA) and in particular α-1,3 and α-1,6 oligomannoside specific plant lectins proved markedly inhibitory to both viruses. The 50% effective concentrations (EC_{50}) of these compounds generally ranked between 1 and 5 μg/ml for both viruses. They were not measurably cytotoxic to the cell cultures at 100 μg/ml. Time-of-addition experiments in which the plant lectins derived from Galanthus nivalis (GNA) and Hippeastrum hybrid (HHA) were added to the FIPV-infected cell cultures at different time points after virus infection, revealed that they act at an early step of the replication cycle, most likely the viral entry process.

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Antiviral Effect of Plant Compounds of the *Alliaceae* Family Against the SARS Coronavirus

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Garlic (*Allium sativum*) and leek (*Allium porrum*) are two members of the *Alliaceae* family and are commonly used as food and herbal supplements with diverse health benefit effects including antiviral activity. Garlic bulbs accumulate several sulfur-containing compounds (including alliin and allylsulfides) and two types of mannose-binding lectins [*Allium sativum* agglutinin I (ASA-I) and ASA-II]. The in vitro antiviral effect of a standardised garlic extract on SARS coronavirus strain Frankfurt 1 (SARS-CoV FFM1) was evaluated by a colorimetric cell proliferation assay. A concentration dependent inhibitory effect was evident when garlic extract was added to Vero E6 cells infected with 100 $CCID_{50}$ of SARS-CoV. The antiviral effect of garlic was further examined by testing the SARS-CoV inhibitory activity of alliin, allylsulfide, allyldisulfide, allylmethylsulfide and the garlic lectins ASA-I and ASA-II. No activity could be demonstrated with either the lectins or the sulfur-containing compounds. However, the in vitro anti-SARS-CoV activity of the Allium porrum agglutinin (APA), an oligomannoside specific lectin encoded by leek, was also investigated. APA was found to inhibit SARS-CoV replication by 50% at a concentration below 1 μg/ml. No measurable cytotoxicity could be demonstrated at 50 μg/ml resulting in a remarkable selectivity index greater then 50 in this cell culture system. Although the effective garlic compound has not yet been identified, we demonstrate in this study that members of the *Alliaceae* family show interesting anti SARS-CoV properties, which in the case of leek are produced by the leek lectin.

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Possibilities to Inhibit the Replication of a Severe Acute Respiratory Syndrome (SARS) Associated Coronavirus

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Recently, a novel coronavirus causing SARS has been identified which spread over 29 countries all over the world thereby infecting more than 8000 people. About 800 people died because of the lack of effective chemotherapeutics. To search for a specific antiviral, we applied a CPE-inhibitory assay and examined the antiviral effect of various natural products and synthetic compounds against the SARS-associated coronavirus isolate FFM1 (SARS-CoV-FFM1) in Vero76 cells. Inhibitors of viral adsorption like pentosan polysulfate, heparin, heparan sulfates, and dispirotripiperazine derivatives led only to very poor if any reduced coronavirus-induced CPE, whereas several phenolic polymers showed a good antiviral activity. In addition, the antibiotic hygromycin B, a water soluble extract horn melissa, and several heterocycles with guanidino or thiaminodino substitutes exhibited a moderate antiviral activity. Moreover, various nucleoside analogues and their *cyclo*Sal-pronucleotides were included in the antiviral tests. While aciclovir, penciclovir, ganciclovir, cidofovir, and ribavirin were found to be inactive, brivudin and some of its *cyclo*Sal derivatives (*cyclo*Sal-BVDUMP) inhibited

the coronavirus induced CPE. These results suggest that *cyclo*Sal-BVDUMP may be potential antiviral compounds for treatment of SARS-CoV infections.

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Influenza-Inhibitory Effects of Viramidine in Cell and Animal Systems

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Viramidine, the 3-carboxamidine derivative of ribavirin, was effective against a spectrum of influenza A (H1N1, H3N2, H5N1) and B viruses in MDCK cells, with EC_{50}'s ranging from 2 to 32 μg/ml; the IC_{50} (50% toxic concentration) of the compound in MDCK cells was approximately 1000 μg/ml. Oral gavage administration to mice infected with influenza A/NWS/33 (H1N1), A/Shangdong/09/93 (H3N2), B/Hong Kong/5/72 or B/Sichuan/379/99 viruses was highly effective in preventing death, lessening decline in arterial oxygen saturation, inhibiting lung consolidation, and reducing lung virus titers. The minimum effective dose in these studies ranged from 15 to 30 mg/kg per day, the maximum tolerated dose being approximately 400 mg/kg per day, when administered twice daily for 5 days. Effective therapy could be started up to 12 h after virus exposure. Efficacy was also seen when viramidine was administered in the drinking water to A/NWS/33 virus-infected mice. A comparison of once, twice, or thrice daily treatments indicated the twice daily regimen to be most effective. Comparisons with ribavirin indicated similar efficacies, but the maximum tolerated dose of ribavirin was approximately 150 mg/kg per day, over two-fold less than that of viramidine.

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Anti-influenza Rimantadine and Novel Phenol-Contained Compounds Efficacy After Transdermal Delivery

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Transdermal drug delivery is a novel method of pharmacotherapy for many diseases that can prolonge drug's action, reducing adverse reactions risk and avoiding concentration hopping. We concidered this method for preventive action and therapy of virus respiratory infections. This work is aimed at studying of the possibility and comparative transdermal delivery estimation for some novel phenol-contained compounds and rimantadine in various doses. Substances were administered in the hydrogel matrix in doses 1 and 2 mg per mouse applied on the shaved back 1 day before infection. Both experimental and control groups were infected intranasally with highly pathogenic influenza virus A/PR/8/34(H1N1); animals deaths were recordered for 14 days. The results of investigation show high anti-influenza activity for both rimantadine (LD_{50} difference between control and experimental group is 1.75–2.0 and 2.75–3.0 $\log_{10}$ for doses 1 and 2 mg per mouse, respectively) and bis-phenol substituted compounds with different "bridge" structures (averaged 1.6 $\log_{10}$ for dose 1 mg/mouse). Accordingly, transdermal delivery of antiviral drugs can be perspective for prophylaxis and treatment of viral deseases.

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Antioxidant Properties of a Plant Preparation with Anti-Influenza Virus Activity

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By three separate and complementary methods—DPPH, β-carotene-linoleic acid and NBT-reduction assays—it was established that a polyphenol-rich extract from the medicinal plant *Geranium sanguineum* L. (PC) with strong anti-influenza virus activity, possessed antioxidant and radical scavenging capacities. For comparative reasons, caffeic acid and the synthetic antioxidant BHT were used. Further, it was demonstrated that the EtOAc fraction retaining the majority of the in vivo protective effect, exhibited a strong O_2^- scavenging activity while the *n*-BuOH fraction, containing the majority of the in vitro antiviral activity, provoked generation of O_2^-. The O_2^- scavenging activity of all three preparations correlated with the rate of their protective effect shown in the lethal experimental A/Aichi influenza virus infection in mice. This infection is accompanied by profound changes in lung tissue metabolism, which lead to intensive generation of reactive oxygen species. It was assumed that the antioxidant properties of PC might contribute to the overall protective effect in vivo.

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Protease Inhibitors from *Streptomyces* Inhibit Influenza Virus Replication

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An extensive screening study for the production of protease inhibitors has been carried out on 90 *Streptomyces* strains; 18 of them (20%) produced inhibitors, which belonged to

the group of serine protease inhibitors. The most active ones, produced by *Streptomyces* sp. 225b (SS-225) with specific inhibitory activity (SIA) of 1544.6 kIU/mg and by *Streptomyces* sp. 34-1 (SS-34) with SIA of 1000.0 kIU/mg were subjected to purification by ion exchange chromatography, gel filtration and HPLC analysis. Further the anti-influenza virus properties of SS-225 and SS-34 were studied, bearing in mind that the virulence of influenza viruses depends on the ability of the precursor HA0 to be cleaved post-translation to subunits HA1 and HA2 by host trypsin-like proteases and the inhibition of this cleavage by exogenous protease inhibitors may result in suppression of viral replication SS-225 and SS-34 exhibited a significant antiviral effect it as selective, strain-specific and dose-related. Most susceptible to inhibition was the penetration of viral particle as well as the late stages of viral replication. All experiments were performed in parallel with the known proteolytic inhibitor ε-amino caproic acid.

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The Identification of Novel Small Molecule Inhibitors of Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is one of the leading causes of all lower respiratory tract infections in infants, children, the elderly and the immunocompromised. A selected set of 20,000 compounds from the Arrow compound collection was evaluated at a single concentration against respiratory syncytial virus (RSV) in a whole cell assay using an XTT colorimetric readout. An initial hit rate of approximately 1% was seen and this hit population yielded 100 compounds with IC_{50}s of less than 50 μM. The confirmed hit list contained analogues from two distinct chemical series which consistently showed activity in the XTT assay. These were a series of quinolines and a series of triazines which exhibited IC_{50}s ranging from 0.5 to 25 μM. In addition, there were a number of compound singletons some of which had IC_{50}s as low as 300 nM. All confirmed hits were further evaluated in secondary ELISA and plaque assays. The triazines and the quinolines both showed good activity in the plaque assay but were less active in the ELISA. One of the singletons showed good activity in the XTT, ELISA and plaque assays. Following an initial hit to lead expansion, this singleton was developed into a series of novel inhibitors, the characterisation of which is described in an accompanying abstract.

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Antiviral Terpenoids From the Chinese Medicinal Plant *Schefflera octophylla*

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Schefflera octophylla (Lour.) Hams (Araliaceae) is a medicinal plant grown in South China. Its traditional uses mainly are anti-pyretic, anti-inflammatory and anti-rheumatic efficacies. Moreover, it is a principal ingredient of an herbal tea widely used in Hong Kong for the treatment of common cold. Recently, we found the aqueous extract of *Schefflera octophylla* possesses potent antiviral activity against respiratory syncytial virus (RSV) with 50% inhibition concentration (IC_{50}) value of 12.5 μg/ml after screening 22 Chinese medicinal herbs using cytopathic effect (CPE) reduction assay. In the present study, we focus our effort on the isolation of antiviral constituents from this plant. CPE reduction assay and tetrazolium salt reduction (MTT) assay are respectively used to test the antiviral activity and cytotocixity of the isolated compounds. Four terpenoid compounds have been isolated and two of them exhibited antiviral activity against RSV with a similar IC_{50} value of 6.25 μg/ml. The chemical structures of the active compounds have been determined using spectroscopic methods in combination with single crystal X-ray analysis.

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VP1 Sequence Analysis and Susceptibility of Laboratory Strains and Clinical Isolates of Coxsackievirus B3 (CVB3) to Pleconaril

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Pleconaril has been shown to inhibit a wide spectrum of rhinoviruses and enteroviruses. However, resistant CVB3 have been described [Groarke et al., J. Infect. Dis. (1999)]. Characteristic for these resistant viruses was a single amino acid substitution in viral capsid protein VP1 (Ile1092Met or Ile1092Leu). Using hybrid viruses, we now demonstrate that Ile1092 indeed defines the susceptible phenotype to pleconaril. After transferring parts of the VP1 region containing Ile1092 of a susceptible laboratory CVB3 variant to the resistant CVB3 strain Nancy, this resistant CVB3 became pieconaril-susceptible. Comparing VP1 amino acid sequences and pleconaril susceptibility of 5 laboratory and 12 clinical CVB3, the previously described data were confirmed. The amino acid substitution Ile1092Leu was detected

in all econaril-resistant CVB3. In addition, our data reveal a natural polymorphism of amino acid position 1092 which need not necessarily by associated with drug resistance. In one of the clinical CVB3 isolates a Ile1092Val was detected. This amino acid substitution did not affect the efficacy of pleconaril treatment. These results confirm that pleconaril resistance can be mapped to amino acid 1092 in VPI which will allow rapid genotypic analysis of pleconaril susceptibility by RT-PCR and sequence analysis.

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Effect of Disoxaril on the Replication of Disoxaril-Sensitive and Disoxaril-Dependent Mutants of Coxsackievirus B1

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The effect of disoxaril on the production of infectious virions during the replicative cycle of the disoxaril-sensitive strain and the disoxaril-dependent mutant of Coxsackievirus B1 obtained by this strain was studied. We used the one-step viral growth cycle experimental setup (multiplicity of infection: ~50).

The results' analysis shows that even the late addition of the compound (i.e. during the exponential phase: 5–6 h after the virus inoculation) guarantees full production of the infectious virions at the end of the one-step cycle: hour 9th. These data lead to the conclusion that structural changes of the capsid protein VP1 in the disoxaril-dependent mutant do not affect the process of uncoating and the following syntesis of viral RNA and proteins. Such changes affect visibly the stage of matured virions' assembling only.

The above findings are in contrast with the observed effect of disoxaril onto the replication of the wild disoxaril-sensitive strain in a similar setup. In this case, the inhibitory effect of the compound strongly depends on the moment of its adding during the latent period (0–3 h after virus inoculation). Furthermore, this effect is strongest at hour 0. Later on, adding the compound throughout the exponential phase does not express whatever significant effect on the production of matured virions. These data are in compliance with the literature references pointing out that disoxaril blocks virus uncoathing (Smith et al., 1986; Zeichhardt et al., 1987; Fox et al., 1991; Diana et al., 1992).

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Substituted Imidazo[4,5-*b*]pyridin-2-ones and -thiones as Inhibitors of Coxsackie B3 Replication

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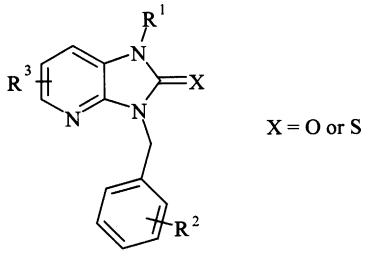
Substituted imidazo[4,5-*b*]pyridin-2-ones and -thiones (X = O or S, R^1 = H or CH_3, R^2 = H or F, R^3 = H, F, Cl, Br, CH_3, OCH_3, etc.) represent a novel class of compounds with activity against the coxsackie B3 virus.



We have synthesized a set of analogues within this class of compounds to get insight into structure-activity relationships. The synthesis and anti-CBV-3 activities of these compounds will be presented.

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Measurement of Neutralizing Antibodies to Interferon-β by an MxA Assay Using ELISA

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Mixovirus resistance protein A (MxA) is an antiviral protein induced exclusively by type I interferons and some viruses, and is therefore an important biological marker for measuring the biological activity of interferons. In order to measure the neutralizing antibodies to interferon from the patients under interferon therapy, we have set up an ELISA assay for measurement of the inhibition of the induction of MxA protein in A549 cells by interferon treatment. The human sera were serially diluted and mixed with 10 IU of interferon-β for neutralizing reaction. The mixture and serially diluted interferon standards were then incubated with the A549 cells. MxA protein is then measured on cell lysates using monoclonal antibodies to MxA. The parameters or reaction conditions of the assay have been optimized. These include

the standard curve, the linearity, specificity, reproducibility, etc.

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Evaluation of Bioavailability of interferons by One-Step Real-Time RT-PCR Assay for Measurement of MxA mRNA Expression

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Interferons induce many proteins in the cells; some are directly involved in the antiviral state. One example of this is MxA proteins, which constitute a family of IFN-α/β-inducible GTPase that display antiviral activity against specific viruses. To evaluate the bioavailability of interferons in multiple sclerosis patients, a one-step real-time RT-PCR assay using Lightcycler for quantitation of the MxA mRNA has been developed. The nucleotide sequences of MxA deposited in the GenBank were aligned and a pair of primers and two hybridization probes were designed based on the conserved regions. The RNA standards were generated by the in vitro transcription from cloned MxA gene in a plasmid vector. The parameters or reaction conditions of the Lightcycler assay have been optimized using A549 cells treated with serially diluted interferon-β. The total RNA was extracted from the cells and quantitatively detected by the lightcycler assay. For evaluation of bioavailability of type I interferons in multiple sclerosis patients, PBMCs were prepared and the total RNA was extracted from PBMC by Trizol. The MxA RNA levels were then quantitated using one-step RT-PCR assay. Currently this assay is being validated with regard to the sensitivity of detection, specificity, precision, accuracy, linearity, range, robustness, and repeatability.

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Fully Size Human Antibodies Against Ebola Virus

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Ebola hemorrhagic fever is the most severe form of viral hemorrhagic fever with the highest mortality rate. Involvement of the antibodies in the mechanism of recovery from filovirus infection was shown in the experiments with hyperimmune horse immunoglobulin, so human antibodies could be valuable resource. Fully human Mab against Ebola virus was constructed from combinatorial Vh and VI domains and constant domains of human IgG1. Variable domains were obtained from combinatorial phage libraries of human scFv fragments (Griffin 1, MRS, England). Obtained of the fully human Mab was produced in 293T human cells, from which the antibody was purified by affine chromatography. Binding activity of this antibody was tested in ELISA, using Ebola and Marburg viruses. Immunoblot analysis confirmed that obtained antibody was specific to Ebola NP and did not bind with Marburg proteins. A standard assay of virus neutralization (PRINT) was performed.

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Protective and Immunomodulating Effect of Proteolysis Inhibitor During Experimental Rabies Infection

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Our previous extensive studies certify the significance of the proteolysis system's role in the physiology of various viruses and pathogenesis of viral infections. We have studied the protective action of the known proteolysis inhibitor—E-aminocaproic acid (E-ACA) during the model infection of the mice, caused by intramuscular infection. The animals of the reference group were injected twice a day with 0.2 ml of 10% E-ACA solution during the day of rabies infection and the four following days. The results show that E-ACA injection demonstrates sufficient protective action. The immunization of the experimental and reference groups of animals were performed intraperitoneally in paired injections with the week-long interval with the inactivated anti-rabies vaccine. The reference group of animals was immunized with the vaccine diluted in 5 and 25 times on the physiological solution and the experimental group—on the 10% solution of E-ACA. On the 8th day after the second immunization, the both groups were infected intracerebrally with the rabies strain CYS, pathogenic for mice. It was found that the same anti-rabies vaccine but diluted on the E-ACA solution acted more efficiently. The results obtained show that the mice immunization accompanied with E-ACA addition raises the level of the anti-rabies defense sufficiently. We propose as the most efficient one, the anti-rabies vaccine modification through the addition of the proteolysis inhibitor which serves as the antiviral agent and immunomodulator.

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Cloning and Sequencing of Variable Domains of Protective Mabs Against Tick-Borne Encephalitis Virus

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Tick-borne encephalitis (TBE) is a viral disease of the central nervous system. The main treatment for TBE is immunoglobulin from human sera, but it is accompanied with the possible biological risk. Chimeric and humanized antibodies could be valuable reagents for therapy. The first step on this way is selection of parental murine monoclonal antibodies (Mabs) which can both neutralize viral infectivity and protect model animals. Mabs with neutralizing activities were selected from a panel of mouse Mabs specific to glycoprotein E of TBE virus. Those Mabs were tested for their protectivity using a suckling-mice model, and five of them were found to be protective. RT-PCR fragments encoding variable domains of the heavy and light chains of the protective Mabs were cloned and sequenced. The following computer analysis of the obtained nucleotide sequences helped to identify their CDRs and to find a strategy for humanization of combinatorial fully human antibodies obtained using phage display technique.

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Identification and Characterization of a Potent and Specific Small Molecule Inhibitor of Human Rotavirus

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Rotaviruses are the single most important cause of severe diarrhea in infants and young children worldwide. Rotavirus infection produces a spectrum of responses that vary from subclinical infection, to mild diarrhea, to severe and fatal dehydrating illness. Rotavirus infections result in more than 140 million cases of diarrhea each year in infants and young children, and 600,000 to 1 million deaths annually worldwide. In the U.S., approximately 3 million children are affected each year, resulting in 775,000 physician visits, 104,000 hospitalizations and about 20 deaths. There is currently no preventative or specific antiviral therapy to combat rotavirus infection and disease. In 1999, the use of Rotashield (rotavirus vaccine) was no longer recommended because of data that indicated a strong association with intussusception (bowel obstruction) among some infants during the first 1–2 weeks following vaccination. In an effort to identify small molecule inhibitors of rotavirus replication, we developed a rotavirus cell based high throughput screening (HTS) assay and screened the entire ViroPharma chemical library. This HTS permitted identification of a compound series with favorable anti-rotavirus activity and pharmacokinetic properties. The lead compound in this series, VP-1147 (MW = 437.92), demonstrated potent (45 nM against WA strain of rotavirus) and specific anti-rotavirus activity, since this compound was inactive against other unrelated viruses. Selection and mapping of drug-resistant variants indicated that the compound target is probably a gene product expressed at the time of virus replication/transcription. VP-1147 represents a potential breakthrough in the discovery of small molecule antivirals directed against human rotaviruses.

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HCV NS3 Protease and NS5B Polymerase Inhibitors as Antiviral Agents

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Specific anti-HCV chemotherapies are required in the treatment of chronic HCV infection, particularly for patients that fail to achieve a sustained response, or who cannot tolerate the adverse effects of current treatment. In an effort to discover novel therapies, two viral enzymes were targeted in our drug discovery effort. The HCV encoded NS3 serine protease and the NS5B RNA-dependent RNA polymerase are enzymes that are essential for viral replication and prime targets in the search for specific anti-HCV chemotherapeutics. The NS3 protease is prone to inhibition by N-terminal cleavage products and a corresponding hexapapetide was optimized through a rational drug-design strategy to a conformationally constrained macrocyclic tripeptide, BILN2061, that effectively reduced HCV RNA plasma levels in a short term study with HCV genotype 1 infected patients. In contrast, a uniquely modified NS5B RNA polymerase construct with a lower affinity than mature NS5B for RNA substrate was subjected to a high-throughput screening campaign to identify a novel class of non-nucleoside polymerase inhibitors. Novel benzimidazole 5-carboxamide derivatives specifically inhibited productive RNA binding to the polymerase. We used the HCV subgenomic replicon to select and characterize NS3 and NS5B mutations that confer resistance to BILN2061, and the benzimidazole 5-carboxamide inhibitors, respectively. The cell-based replicon system demonstrated that a polymerase inhibitor retains activity against BILN2061-resistant replicons. These two classes of inhibitors are complementary in cell culture models of HCV RNA replication and expand the repository of potential treatments of chronic HCV infection.

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Inhibition of Hepatitis C Virus RNA Replication by Benzimidazoles with High Affinity for the 5′-Untranslated Region of the Genomic RNA

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Domain II of the 5′-untranslated region (UTR) of the hepatitis C virus (HCV) genomic RNA has been shown to be essential for replication of viral RNA, and therefore is an attractive drug target for development of chemotherapies for HCV infections. Ligands that bind weakly, but selectively to a specific region of the domain II region have been identified in a high throughput screen employing mass spectrometry (MS)-based methodology. Subsequent structure–activity relationship (SAR) studies and optimization efforts guided by the MS assay method have afforded ligands with high affinity and selectivity for the domain II RNA target. Members of this series inhibit HCV RNA replication in cellular assays at low micromolar concentrations, and are not cytotoxic at concentrations up to 100 μM. A representative compound exhibited a favorable preliminary pharmacokinetic profile, including oral bioavailability. This class of ligands demonstrates that RNA is a tractable target for HCV drug discovery, and that the described methods can be used to generate leads.

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Nucleoside Inhibitors of HCV Replication and the Corresponding Nucleoside Triphosphate Inhibitors as Chain Terminators of HCV NS5B Polymerase

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Nucleoside analogs are proven prodrugs to treat viral infections. The effectiveness of this class of compounds is mainly derived from the ability of the corresponding nucleoside triphosphate NTP) to be incorporated by viral polymerases and cause chain termination resulting in inhibition of viral replication. We have recently discovered nucleoside compounds that are potent inhibitors of HCV replication in a cell-based replicon system. The corresponding nucleoside triphosphates are potent inhibitors of the HCV RNA-dependent RNA polymerase (NS 5B). Investigation of the mechanism of inhibition by the NTP compounds shows that they are kinetically competitive against a natural NTP with a similar nucleobase and non-competitive against NTP with dissimilar bases. Additional experiments demonstrate that they serve as alternative substrates in place of a natural NTP in HCV NS5B-catalysed RNA-dependent RNA polymerization. Once incorporated, they efficiently prevent the elongation of the growing chain. The mechanism of chain termination cannot be due to a chemical inability to extend, since these inhibitors contain a free hydroxyl group at the 3′-position of the ribose ring. Modeling studies suggest that chain termination may instead be due to physical inaccessibility of the terminal hydroxyl to the subsequent incoming nucleotide.

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In Vitro and In Vivo Evaluation of HCV Polymerase Inhibitors as Potential Drug Candidates for Treatment of Chronic Hepatitis C Infection

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Major hurdles for the development of effective new therapies for chronic HCV infection are the lack of suitable in vitro and in vivo assays. We have utilized a unique cell-based assay and our HCV Trimera^{TM} mouse model to evaluate drug candidates (HCV polymerase inhibitors) as a potential therapy against chronic hepatitis C. Two different molecules were designed, synthesized, and tested for their ability to inhibit the activity of the HCV polymerase. In a biochemical assay, they both showed good inhibition activity ($IC_{50} < 0.1\,\mu g/ml$). The first molecule, BC2125 is water soluble with a good rodent oral PK profile ($T_{max} = 4.0\,h$ and $C_{max} \sim 5\,\mu g/ml$ for 50 mg/kg in rats). This molecule was proved to be non-cytotoxic ($CC_{50} \sim 100\,\mu g/ml$ for HepG2, FLC4 and Huh7 cell-lines). It also showed inhibition of IICV replication at concentrations of 2–10 μg/ml using our cell-based assay for both Huh7 and FLC4 cells. In vivo, BC2125 was able to reduce viral loads and percentages of HCV-positive mice in HCV Trimera^{TM} mice at 100, 50, and 30 mg/kg per day (treatment p.o., b.i.d. for 8 days). These reductions were dose dependent and statistically significant. Fourteen days oral toxicity study in mice showed no toxicity signs (necropsy, histopathology, weight loss) for 50 or 250 mg/kg per day. The second molecule, BC2329 was administered orally, as a suspension in water, to rats and gave T_{max} of ∼0.5 h and C_{max} of $\sim 30\,\mu g/ml$ for 50 mg/kg. The molecule was proved to be non-cytotoxic ($CC_{50} \sim 200\,\mu g/ml$ for HepG2, FLC4, and Huh7 cells) and demonstrated HCV inhibition in the cell-based assay for both Huh7 and FLC4 cells ($IC_{50} \sim 5$–$10\,\mu g/ml$). This molecule is currently under evaluation in vivo using the HCV-Trimera mouse model. Good in vitro and in vivo toxicity profiles and PK data together with antiviral inhibitory activity in both assay systems indicate the potential of BC2125 as oral drug candidate for treatment of chronic hepatitis C.

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Susceptibility of Different Genotypes of Hepatitis C Virus to Inhibition by Nucleoside and Non-nucleoside Inhibitors

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Approved chemotherapies for the treatment of hepatitis C virus (HCV) infection consist of combinations of interferon alpha and ribavirin which are efficacious in achieving a sustained viral response in approximately 40–60% of patients, but improved therapies are needed. Optimally, newly developed therapies for HCV infection based on direct inhibitors of HCV enzymes will be broadly effective against all viral genotypes. Recently, several structurally distinct classes of inhibitor of the HCV RNA-dependent RNA polymerase (RdRp) have been disclosed in the literature, including both nucleoside analogues and non-nucleoside compounds. In most cases the inhibitory activity of these compounds has been demonstrated only for the cell-based replicon assay and recombinant RdRp derived from HCV genotype 1b. We have cloned, expressed and purified RdRp's from HCV genotypes 1a, 1b, 2a, 2b, 3a, 4a and 6a. Measurement of the inhibitory potencies of several structural classes of inhibitor reveals differences in the susceptibility to inhibition exhibited by the various genotype RdRps. Inhibitors directed at the enzyme active site retain potency against the RdRps from the different HCV genotypes to a much greater degree than do inhibitors that bind at sites that are physically distinct from the enzyme active site. For some inhibitors, amino acid differences between genotypes 1b and 2b have been identified that contribute to the loss of inhibitory potency. These results have implications for the utility of various classes of inhibitors in treating existing viral variants and for the likelihood of the development of drug resistance during therapy.

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Comparison of Two Anti-HCV Clinical Candidates, VX-950 and BILN 2061, for Potency and Resistance in the HCV Replicon System and in a Novel HCV Protease Animal Model

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VX-950, discovered using structure-based drug design, is a potent small molecule inhibitor of HCV NS3·4A protease and is our first direct antiviral clinical candidate for hepatitis C. VX-950 has a two-step enzymatic mechanism of inhibition where the steady state binding constant and half-life for the most stable enzyme-inhibitor (EI*) complex is 3 nM and 1 h, respectively. In contrast, BILN 2061, a peptidomimetic inhibitor of NS3·4A protease from Boeringher Ingelheim, for which proof of concept has been shown in a 2-day clinical trial, has a one step mechanism of inhibition and a short EI half-life. Potent, sustained antiviral activity has been demonstrated for VX-950 in the HCV replicon system, in which viral RNA could be cleared without rebound after withdrawal of the inhibitor. These studies revealed that VX-950 and BILN 2061 have comparable potency in a long-term viral clearance assay. A comparison of VX-950 and BILN 2061 in a novel mouse model for HCV protease activity will be shown. In addition, distinct, dominant mutations with different resistant mechanisms have been identified for both inhibitors using the replicon system.

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Gene Expression Patterns During Anti-HCV Treatment with Interferons and Interferon Combinations in the HCV Replicon Model

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Types I (α, β, ω) and Type II (γ) interferons (IFN) have been shown to be an effective inhibitors of HCV replication in both infected patients and in HCV replicon culture models. Due to the variety of intracellular targets and pathways regulated by these antiviral agents, differential (and some overlapping) mechanisms are likely involved in the intracellular control of HCV. Previously, we characterized the activity of monotherapy and combination therapy with the major classes of human interferons against HCV replication in an HCV replicon model. Type I interferons generally exhibited antagonism in combination, while Type I/II interferon combinations acted in a cooperative manner against HCV replication. Microarray analysis using two different arrays [1200 member signal transduction array (NIH, NIA); 5544 member GF-200 (Research Genetics, Inc.)] revealed distinct differences in gene expression patterns related to treatment with the different interferons. Multi-Dimensional Scaling Analysis demonstrated that overall gene expression patterns of αIFN and βIFN were highly similar and distinct from that induced by γIFN. Gene expression patterns induced by ωIFN were distinct from that of the other three interferons, but were most closely related to γIFN than αIFN or βIFN. Although the αIFN/γIFN and αIFN/ωIFN combinations exhibited distinctly different types of interactions (synergistic versus antagonistic), overall gene expression patterns for these two treatments were highly similar with only 13/56 up-regulated and 3/45 down-regulated genes showing differential patterns of expression. Gene expression patterns induced by αIFN monotherapy were largely overridden in combination treatments, with expression patterns related to the second interferon being clearly dominant. For example, 5 genes in the MAPK signaling pathway were up-regulated by αIFN monotherapy, but not by the other treatments. All of the interferon treatments appeared to universally down-regulate various components of the cellular pathways involved with apoptosis and programmed cell death. Other genes known to be involved in cellular antiviral responses (e.g. defensin; interferon-inducible protein) were universally up-regulated. Analysis of gene expression patterns during different interferon treatments provides a platform to identify common and differential cellular antiviral mechanisms.

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A Novel Highly Selective Inhibitor of Pestivirus Replication that Targets the Viral RNA Dependent RNA Polymerase

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We report on the highly potent and selective antipestivirus activity of a novel small molecule. Compound "22" inhibited bovine diarrhea virus (BVDV) (various strains of BVDV-1 and BVDV-2) and Classical Swine Fever Virus (CSFV)-induced cytopathic effect in a concentration-dependent manner with an EC_{50} value of 0.006 μg/ml. This activity was paralleled by a similar reduction of BVDV RNA synthesis in MDBK cells, as was quantified by real time Q-RT-PCR. The selectivity index (EC_{50}/CC_{50}) amounted to 10,000. Detailed time of drug addition studies (as assessed by monitoring viral RNA synthesis in one single replication cycle) revealed that the compound inhibits BVDV replication at a time point that coincides with the onset of intracellular viral RNA synthesis. Drug resistant mutants were generated by culturing BVDV (strain NADL) in increasing drug concentrations. Resistant virus, that was at least 100-fold less susceptible to compound "22" than the wild-type virus, carried a F2245 mutation in the viral RNA dependent RNA polymerase (4 out of 4 plaque purified virus isolates). No other mutations were detected in the entire coding region of the drug-resistant virus. Reintroduction of the F2245 mutation in the wild-type

BVDV genome yielded again the drug-resistant phenotype. Yet compound "22" did not inhibit the purified BVDV polymerase. However, the drug efficiently inhibited the activity of the replication complexes (RC) isolated from MDBK cells that had been infected with wild-type virus, but not the activity of RCs that were obtained from MDBK cells that had been infected with the drug-resistant virus.

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Chimeric Yellow Fever-Dengue Tetravalent Vaccine (ChimeriVax™-DEN1-4): Precilnical Safety and Efficacy in Non-human Primates

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ChimeriVax™-DEN1-4 tetravalent vaccine consists of four live, attenuated recombinant virus vaccine strains prepared by excision of the prM and E genes of yellow fever 17D virus and substitution with the gene donors (prME) from low-passage human isolates of DEN1-4 virus stocks. Preclinical studies in vitro (Vero cells) and in vivo (mouse and monkey models) demonstrated that plaque-purified stocks of the tetravalent vaccine are replication competent; genetically stable up to P20 in Vero cells; and do not become more neurovirulent upon extensive in vitro passages. Safety of a tetravalent vaccine mixture was determined and compared to that of YF-VAX® in a formal monkey toxicology test. The lesions produced with tetravalent ChimeriVax™-DEN vaccine were significantly less severe than those with YF-VAX® vaccine. Immunogenicity and protective efficacy of four different tetravalent formulations were measured in 24 cynomolgus monkeys, following a single-dose subcutaneous vaccination and a challenge inoculation 6 months later. All monkeys developed low levels of viremia post-immunization, and 22/24 were fully protected (lack of viremia) when challenged with heterologous wild-type dengue viruses. This demonstrates not only the safety of a recombinant dengue tetravalent vaccine in a formal toxicology test, but also its protective efficacy in a monkey challenge model.

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Are Cholinesterases Inhibited by *cyclo*Sal Nucleotides?

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The *cyclo*Sal pronucleotides are effective Systems for an intracellular delivery of therapeutically active nucleoside monophosphates. Because the *cyclo*Sal concept involves a chemically hydrolysable organophosphate moiety, an interaction with cholinesterases cannot be excluded. Therefore, a study concerning a possible inhibition of cholinesterases by *cyclo*Sal nucleotides have been conducted. Two different cholinesterases are relevant in humans: the highly specific and physiologically important acetylcholinesterase (AChE, E.C. 3.1.1.7) and the unspecific serum butyrylcholinesterase (BChE, E.C. 3.1.1.8) of unknown physiological role. A cholinesterase assay based on the method of Rappaport et al. was established. It was shown that all *cyclo*Sal-phosphate triesters tested in this study were *not* inhibitory against purified human AChE and AChE from *electrophorus electricus*. However, experiments using BChE containing human serum as well as purified human BChE showed inhibition of the enzyme in some cases. Structure–activity relationship studies showed a strong dependence on the chemical structure of the pronucleotide. As BChE inhibition would be a potential unwanted side effect in the possible therapeutic application of *cyclo*Sal nucleotides, ways to avoid this effect have been worked out. Therefore, the influence of the stereochemistry at the phosphorous atom was determined, and novel substitution patterns in the aromatic ring were studied. The synthesis, characterization and in vitro antiviral evaluation of some of these new pronucleotides will be presented. As a conclusion of the investigations clear structural characteristics have been identified to avoid interaction of the *cyclo*Sal-pronucleotides even with BChE and will be presented. These new compounds still selectively deliver the nucleotides and thus conceptionally achieve a successful thymidine-kinase bypass.

Oral Session VII: Late Breaker Presentations

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PG 301029 Inhibits the HCV Surrogate BVDV Through a Novel Late Stage Mechanism of Action

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The compound PG 301029 was identified as an effective inhibitor of the replication of bovine viral diarrhea virus (BVDV). Subsequent research has defined the novel mechanism of antiviral action of this compound and the possibility of using the compound in combination with ribavirin or as a supplement to the existing therapeutic strategy of ribavirin plus interferon. Our research on the antiviral properties of PG 301029 has effectively demonstrated its efficacy and novelty as an inhibitor of BVDV and yielded a strong rationale for the clinical development of the compound. The specific antiviral properties of PG 301029 include the following: (1) In cell-based in vitro assays PG 301029 was a highly effective inhibitor of BVDV, exhibiting IC_{50} values of approximately 0.65 μg/ml. (2) PG 301029 was 100- to 200-fold less toxic to the host cells infected with BVDV than ribavirin and exhibited a much greater therapeutic index. (3) PG 301029 was nontoxic in fresh human hepatocytes at the highest concentrations tested (316 μg/ml), which is a concentration nearly 500-fold greater than concentrations determined to be efficacious against BVDV. (4) Mechanism of action studies revealed that PG 301029 inhibits steps in the virus replication cycle that result in greatly reduced levels of viral RNA synthesis. (5) The inhibitory activity of PG 301029 did not appear to be a result of inhibition of virus entry, translation initiation at the internal ribosome entry site (IRES) or the activity of NS2/3 viral protease. (6) In preliminary studies, PG 301029 did not appear to rapidly select for a drug-resistant BVDV strain. (7) In combination anti-BVDV assays, the addition of PG 301029 to ribavirin appeared to yield a reduction in the toxicity of ribavirin, suggesting a potential therapeutic benefit to the combination of compounds. Our data suggest that PG 301029 exhibits a novel antiviral mechanism of action that is distinct from that of existing therapies. The ability of PG 301029 to effectively inhibit HCV and BVDV replication would provide benefits over existing therapies with ribavirin and the addition of PG 301029 to existing therapies of ribavirin plus interferon may be beneficial.

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Identification of Antiviral Nucleoside Analog Metabolizing Enzymes Through Bioinformatic Data Mining

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The benzimidazole ribonucleoside BDCRB has been identified as a potent HCMV inhibitor, but in vivo testing revealed poor bioavailability due to extensive glycosidic bond cleavage. This discovery prompted the development of 1263W94 (maribavir)—an L-ribofuranosyl analog with greatly enhanced bioavailability. As there is urgent need to develop nucleoside analog metabolism screening systems, the aim of this study was to determine why BDCRB undergoes more extensive glycosidic bond cleavage than maribavir. We first determined initial cleavage rate in two first-pass models—human liver microsomes and Caco-2 colon carcinoma whole cell homogenates. Both systems rapidly cleaved BDCRB but not maribavir. BDCRB cleavage by microsomes was completely inhibited by pre-incubation with troleandomycin but not other inhibitors, suggesting that CYP3A4 was responsible. CYP3A4, however, is known to be absent from Caco-2 cells. We consequently employed Genechip technology to identify additional BDCRB-metabolizing enzymes. Affymetrix U95A genechips were utilized to quantitate relative mRNA expression levels in human duodenum, jejunum, ileum, colon and Caco-2 cells. These chips contained 40 probe sets corresponding to human glycosylases and enzymes known to cleave N-glycosidic bonds. Expression data and reported mechanisms led to selection of four enzymes, which were assessed for BDCRB and maribavir glycosidic bond cleavage. 3-Methylpurine DNA glycosylase II cleaved BDCRB roughly sixfold faster than maribavir, and 8-oxoguanine DNA glycosylase was roughly threefold faster. The initial rate of BDCRB cleavage by purine nucleoside phosphorylase was faster than maribavir as well. tRNA-guanine transglycosylase, however, did not cleave BDCRB or maribavir. Thus, in addition to CYP3A4 we have identified three enzymes responsible for selective BDCRB glycosidic bond cleavage. Of further interest is that glycosylases are known to be localized in the nucleus, a common destination of antiviral therapeutics. Their implication in drug metabolism may present a new obstacle to antiviral drug delivery.

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Antiviral Action of Interferons on Human Coronavirus

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Severe Acute Respiratory Syndrome (SARS) has been associated to a newly discovered member of Coronaviridae (SARS-CoV). Efforts are being made to identify specific treatment for SARS but at the moment unsuccessfully. In this context, the inhibitory effect of different types of Interferon (IFN) on SARS-CoV replications was evaluated. The results suggest that SARS-CoV is rather resistant to the action on natural IFN alpha and IFN gamma and weakly sensitive to IFN beta. Interestingly, the modest activity by natural IFN alpha is not shown by recombinant IFN alpha, subtypes 1, 2b, 5, 6, 7a, and 8b applied singularly even at exceedingly high concentration. Although IFN beta seems to be the most potent among the IFNs tested, its IC_{50} values against SARS-CoV is much higher as compared to IC_{50} values calculated versus the other viruses tested (EMC, VSV, and NDV). However, when IFN beta and gamma are applied in combination they show a synergistic antiviral effect depending upon the concentration of IFN gamma used in combination with IFN beta. In order to characterize the specific mechanism by which IFN beta and IFN gamma inhibit synergistically SARS-CoV replication in Vero cell cultures we analysed the expression of IFN-induced MXA and PKR. The results showed that while the changes in mRNA-MXA levels paralleled the changes in the levels of the antiviral state induced by a mixture of IFN beta/gamma the same did not occur when the two IFNs were applied individually, suggesting that the induction of the mRNA-MXA does not contribute to the level of the antiviral state. Study are in progress to further characterize the antiviral activities of IFNs on SARS-CoV and to assess the duration of the antiviral state induced by IFN. This issue will be important in order to evaluate the possibility to use IFN in prophylaxis rather than in treatment of SARS.

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Integration of Clinical Data, Pathology, and cDNA Arrays in Influenza-Infected Pigtail Macaques: A Novel Approach for Understanding the Genetic Basis of Virulence

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For most severe pandemics, the exact contribution of individual genes to virulence is still largely misunderstood. A necessary step toward that understanding is characterization and integration in the clinical and pathological context of gene expression regulation during infections with non-pandemic strains. Then, introduction of one or several genes from a pandemic strain into a non-pandemic influenza virus can be evaluated at the higher resolution provided by cDNA arrays and the specific effects of introduced genes on pathogenesis accurately singled out. **Hypothesis and Approach:** We conducted a pilot study by infecting Pigtail macaques with a reconstructed strain of human influenza A/Texas/36/91, and hypothesized that these animals would not only respond to the human strain similarly to humans, but that gene expression patterns in lungs and tracheobronchial lymph nodes would fit into a coherent and complete picture of the host–virus interactions during infection. **Results and Conclusions:** The disease observed in infected macaques was indeed a good match for the human counterpart. Clinical signs and acute antibody titer appeared with induction of interferon and B cell activation pathways, respectively. Transcriptional activation of neutrophils, monocyte-macrophages, and T cells coincided with histopathological evidence of innate and adaptive immune responses and apoptotic pathway regulation with local cellular reaction. Additionally, arrays suggested that genetic recovery preceded gross tissue recovery and offered new evidence of the importance of cytotoxic T cells and natural killer cells throughout infection. Consequently, this non-human primate model of influenza promises to significantly enhance our knowledge of non-pandemic and later pandemic human influenza infection at the genetic level, and help in the development of targeted prophylactics and therapeutics.

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Novel Small Molecule Inhibitors of Rhinovirus Replication that Target the HRV 2B Nonstructural Protein

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A cell-based screen was used to identify inhibitors of human rhinovirus 16 (HRV-16) infection. While most of the screen hits acted at the initial stages of infection, virus binding and entry, several targeted post-entry steps of viral replication. One such compound was a novel tri-substituted triamino triazine that displayed potent ($EC_{50} \sim 0.02\,\mu M$) and selective ($CC_{50} \sim 54\,\mu M$) inhibition of HRV-16. Activity was limited to the replication stage of infection as uncoating, initial translation, and polyprotein processing were unaffected, but viral RNA synthesis, host shutoff and progeny release were restricted. The compound was selectively more potent against a representative panel of 16 of the species A HRVs (average EC_{50} $0.1\,\mu M$) than it was against a panel of 12 species B HRVs (average EC_{50} $1.6\,\mu M$). Varied substitutions around the triazine, however, enhanced coverage of both groups of viruses. Highly resistant viruses were selected by passage in increasing inhibitor concentrations, confirming a virus-based target. Nucleotide sequence analysis identified unique substitutions within the HRV-16 (species A) and HRV-14 (species B) genomes, which conferred resistance upon transfer into a wild-type background. Although resistance mutations varied with respect to species and compound, they invariably mapped to a 34 amino acid region within the 95–97 residue 2B nonstructural protein. These studies reveal a novel series of HRV replication inhibitors and suggest that the HRV 2B protein is a potential target for antiviral therapy.

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Barcelona, Spain
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The purpose of the International Conference on Antiviral Research is to provide an interdisciplinary forum at which investigators involved in basic, applied, and clinical research worldwide can meet to review recent developments in all areas of antiviral research. Specific topics to be covered in the program include synthesis and chemistry, biochemistry and mechanism of action, molecular biology and drug targeting, 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.

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