ISAR
INTERNATIONAL SOCIETY
FOR ANTIVIRAL RESEARCH

Program and Abstracts *of the* 26th International Conference *on* Antiviral Research (ICAR)

SAN FRANCISCO, CALIFORNIA | May 11th-15th, 2013

ISAR
INTERNATIONAL SOCIETY
FOR ANTIVIRAL RESEARCH

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- Gertrude Elion Award Lecture
- Oral Session 2: Hepatitis Viruses and HIV
- Poster Session 1: Retroviruses, Hepatitis Viruses, Respiratory Viruses, and Antiviral Methods

Monday, May 13, 2013

- William Prusoff Young Investigator Award
- Oral Session 3: Mini-Symposium – Strategies and Tactics in Drug Design
- Oral Session 4: Influenza and Respiratory Infections
- Poster Session 2: Herpesviruses, Poxviruses, Enteroviruses, Emerging Viruses, Other Antiviral Agents and Medicinal Chemistry

Tuesday, May 14, 2013

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International Society for Antiviral Research and Twenty-Sixth International Conference on Antiviral Research

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The International Society For Antiviral Research (ISAR)

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

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INTERNATIONAL SOCIETY FOR ANTIVIRAL RESEARCH

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to the 26th International Conference on Antiviral Research

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Program Highlights

Keynote Address

"Know Thine Enemy: Using Virology and Immunology to Develop a Multifaceted Approach to Dengue Antivirals"

Eva Harris, Ph.D.

Saturday, May 11, 2013
4:15 pm – 5:30 pm

Mini-Symposia

Legacy of Tony Holý: Nucleotides in the Treatment and Prevention of Chronic Viral Infections

Sunday, May 12, 2013
8:15 am – 12:00 pm

Strategies and Tactics in Drug Design

Monday, May 13, 2013
8:45 am – 11:45 am

Prodrugs as a Tool in Drug Discovery and Development

Wednesday, May 15, 2013
8:30 am – 11:30 am

Clinical Symposium

Wednesday, May 15, 2013
1:00 pm – 3:30 pm

Networking Events

Opening Reception

with light hors d'oeuvres

Saturday, May 11, 2013
5:30 pm– 7:30 pm
Location: Atrium 2-5

New Member and First Time Attendee Networking Event

Sunday, May 12
6:30 pm – 7:30 pm
Location: Grand/Market Street Foyer

Career Happy Hour

Monday, May 13
6:30 pm – 8:00 pm
Location: Sens Restaurant
4 Embarcadero Center, Promenade Level
just outside of the Hyatt on the Lobby Level

Women in Science Lunch*

Tuesday, May 14
12:30 pm – 2:30 pm
Location: Seacliff D

Conference Banquet

Wednesday May 15, 2013
Reception 7:00 pm
Dinner 7:30 pm – 9:30 pm
Location: Grand Ballroom

**space is limited and pre-registration is required. Please see the ICAR Registration Desk if you wish to attend or cancel your registration.*

Agenda at a Glance

Saturday • May 11, 2013

Interactive Workshop: Drug Discovery and Development 101

1:30 pm – 2:00 pm
- **Christos Petropolous, Ph.D.** *(LabCorp/Monogram Biosciences)* – Will be discussing the requirements for resistance testing in drug development and the use of resistance data to define a compound's mechanism of action.

2:00 pm – 2:30 pm
- **James M. McKim, Ph.D.,** *DABT (CeeTox, Inc.)* – "An *In Vitro* Model for Identifying Heart and Liver Specific Toxicity of Antiviral Drugs"

2:30 pm – 3:00 pm
- **Anthony Ham, Ph.D.** *(ImQuest BioSciences)* –"The Importance and Role of Formulation in Antiviral Drug Development Programs"

3:00 pm – 3:30 pm
- **Martine Krauss, Ph.D.** *(Tobira Therapeutics)* "Regulatory Milestones and Requirements in Antiviral Drug Development"

3:30 pm – 3:45 pm **Panel Discussion**

Break

4:15 pm – 5:30 pm **Keynote Address: "Know Thine Enemy: Using Virology and Immunology to Develop a Multifaceted Approach to Dengue Antivirals"**
- **KEYNOTE SPEAKER: Eva Harris, Ph.D.** – *Professor of Infectious Diseases at UC Berkeley, and President of the Sustainable Sciences Institute.*

5:30 pm – 7:30 pm **Opening Reception**

Sunday • May 12, 2013

7:15 am – 8:15 am **Continental Breakfast**

Oral Session I: Mini-Symposium: "Legacy of Tony Holý: Nucleotides in the Treatment and Prevention of Chronic Viral Infections"

8:15 am – 8:30 am
- **Erik DeClercq, Ph.D., M.D.** *(Rega Institute)* –Personal Note on the Contributions and Legacy of Tony Holý.

8:30 am – 9:00 am
- **John Martin, Ph.D.** *(Gilead)* –Keynote Talk: A Tribute to Antonin Holý

9:00 am – 9:30 am
- **Robert Schooley, M.D.** *(UCSD)* – "Tenofovir in the Treatment of HIV Infection."

9:30 am – 10:00 am
- **Robert Grant, M.D.** *(UCSF)* –"Tenofovir in the Prevention of HIV Infection."

Break

10:30 am – 11:00 am
- **Henry Chan, Ph.D.** *(The Chinese University of Hong Kong)* — "Nucleotide Analogue in Chronic Hepatitis B – From Hope to Reality"

11:00 am – 11:30 am
- **Richard Whitley, M.D.** *(UAB)* –"Nucleotides in the Treatment and Prophylaxis of Herpes and Other DNA VirusInfection."

11:30am – 12:00 pm
- **Tomas Cihlar, Ph.D.** *(Gilead)* – "Future Potential and Therapeutic Opportunities for Nucleoside Phosphonates."

Lunch on Your Own

1:30 pm – 2:15 pm **Gertrude Elion Award Lecture**

2:15 pm – 4:30 pm **Oral Session II: Hepatitis Viruses and HIV**

- **Abstract Speakers**

4:30 pm – 6:30 pm **Poster Session I: Retroviruses, Hepatitis Viruses, Respiratory Viruses and Antiviral Methods**

6:30 pm **New Member and First Time Attendee Networking Event**

Monday • May 13, 2013

7:00 am – 8:00 am **Continental Breakfast**

8:00 am – 8:45 am **William Prusoff Young Investigator Award**

Oral Session III: Mini Chemistry Symposium–Strategies and Tactics in Drug Design

8:45 am – 9:15 am
- **Ernesto Freire, Ph.D.** (John Hopkins) – "A Thermodynamic Approach to the Development of Novel Antivirals"

9:15 am – 9:45 am
- **Daniel Cheney, Ph.D.** (Bristol Myers Squibb) – "Molecular Recognition in Drug Design"

9:45 am – 10:15 am
- **Andrew Woodhead, Ph.D.** *(Astex)* – "Discovery of a Novel Allosteric Inhibitor of the HCV NS3/4a Protein Using Fragment Screening and Structure-Based Design"

Break

10:45 am – 11:15 am
- **John Kadow, Ph.D.** *(Bristol Myers Squibb)* – "The Discovery of an Allosteric NS5B Replicase Inhibitor for the Treatment of Hepatitis C Virus Infection"

11:15am – 11:45am
- **Nigel Liverton, Ph.D.** *(Merck)* – "Discovery of MK-5172, a Macrocyclic Hepatitis C Virus NS3/4a Protease Inhibitor"

Lunch on Your Own

Oral Session IV: Influenza and Respiratory Infections

1:30 pm – 2:00 pm
- **Bruno Canard, Ph.D.** – "RNA Synthesis, Capping and Repair in (+)RNA Viruses: Novel Targets for Drug Design"

2:00 pm – 4:30 pm **Abstract Speakers**

4:30 pm – 6:30 pm **Poster Session II: Herpesviruses, Poxviruses, Enteroviruses, Emerging Viruses, Other Antiviral Agents and Medicinal Chemistry**

6:30 pm – 8:00 pm **Career Happy Hour**

Tuesday • May 14, 2013

7:30 am – 8:30 am **Continental Breakfast**

8:30 am – 10:00 am **Oral Session V: Herpesviruses, Poxviruses, Other Antiviral Agents**

- **Abstract Speakers**

10:00 am – 10:30 am **Break**

10:30 am – 12:30 pm **Oral Session VI: Emerging Infections**

12:30 pm – 2:30 pm **Women in Science Roundtable*:** This session will address the challenges and opportunities encountered by female scientists while navigating the twists and turns of career progression in todays' environment. Come talk to scientists in the industry, government and academic fields.

**Please note registration is limited for this event due to room capacity.*

Wednesday • May 15, 2013

7:30 am – 8:30 am **Continental Breakfast**

Oral Session VII: Mini Chemistry Symposium – Prodrugs as a Tool in Drug Discovery and Development.

8:30 am – 9:00 am
- **Valentino Stella, Ph.D.** *(University Kansas)* – "A Case for Prodrugs"

9:00 am – 9:30 am
- **Randall Lanier, Ph.D.** *(Chimerix)* – "Rational Design of Nucleoside Phosphonates for Intracellular Delivery Using Lipid Conjugation"

9:30 am – 10:00 am
- **John Kadow, Ph.D.** *(Bristol Myers Squibb)* – "Successful Application of Phosphate Prodrug Methodology to an HIV Attachment Inhibitor for the Treatment of HIV-1 Infection"

Break

10:30 am – 11:00 am
- **Richard Mackman, Ph.D.** *(Gilead Sciences)* – "Design and SAR of Amidate Prodrugs for Acyclic and Cyclic Nucleoside Phosphonate Antivirals – the Discovery of GS-7340 and GS-9131"

11:00 am – 11:30 am
- **Mike Sofia, Ph.D.** *(OnCore Biopharma)* – "Prodrugs of Nucleosides and Nucleotides for the Treatment of HCV Infection – an Overview"

Lunch on Your Own

12:30 pm – 1:00 pm **Business Meeting**

Oral Session VIII: Clinical Symposium

1:00 pm – 1:30 pm
- **Hetal Kocinsky, M.D.** *(Achillion Pharmaceuticals)* – Clinical Development of Sovaprevir and ACH-3102: Two 2nd Generation Direct-Acting Anti-HCV Agents

1:30 pm – 2:00 pm
- **Amy Patick, Ph.D.** *(Santaris Pharma)* – "Preclinical and Clinical Studies of Miravirsen, a Novel Anti-HCV Therapeutic Targeting the Host Factor Mir-122"

2:00 pm – 2:30 pm
- **John Fry** *(Alios BioPharma)* – "ALS-2200/Vx-135, and the Role of Nucleoside Analogs in the Treatment of Chronic Hepatitis C"

Agenda at a Glance

2:30 pm – 3:00 pm	■ **Eric Lefebvre, M.D.** *(Tobira Therapeutics)* – "Cenicriviroc, a Novel, Once-Daily, Potent Dual CCR5 and CCR2 Antagonist Under Investigation for Treatment of HIV Infection"
3:00 pm – 3:15 pm	■ **Calvin Cohen, M.D.** *(Community Research Initiative of New England)* – "Star Study: Single Tablet Regimen Rilpivirine/Emtricitabine/Tenofovir DF is Non-inferior to Efavirenz/ Emtricitabine/Tenofovir DF in ART-Naïve Adults"
3:15 pm – 3:30 pm	■ **Randall Lanier Ph.D.** *(Chimerix Inc.)* – "CMV Resistance Profile of CMX001"
4:00 pm – 5:00 pm	**Oral Session IX: Late Breaker and Shotgun Poster Presentations** ■ **Abstract and Poster Speakers**
7:30 pm – 9:30 pm	**Closing Banquet**

ICAR Career Happy Hour

SENS RESTAURANT • 4 Embarcadero Center, Promenade Level

Just outside of the Hyatt Regency on the Lobby Level

Monday, May 13
6:30 pm – 8:00 pm

Refreshment will be provided

Focus

This year we will again host an excellent group of moderators who are recognized experts in different areas of antiviral research and have pursued successful careers in academia, government, or industry. In informal group discussions, the moderators are ready to share experience about their career path, answer questions, and provide feedback.

Registration

Please sign up at the ICAR registration desk before 4 pm on Sunday, May 12.

Sectors

Academia
Government
Small Biotech
Mid-size Pharma
Large Pharma
Contract Research Organizations

Program Schedule

Saturday, May 11, 2013

INTERACTIVE WORKSHOP: DRUG DISCOVERY AND DEVELOPMENT 101

Chair(s): Robert Buckheit, Ph.D., Joseph Colacino, Ph.D., and Phil Furman, Ph.D.

GRAND BALLROOM BC

1:30 pm – 3:45 pm

1:30 pm **1. The History, Evolution and Future of Drug Resistance Testing in Antiviral Drug Development.**
Christos Petropolous, Ph.D.
LabCorp/Monogram Biosciences

2:00 pm **2. An *In Vitro* Model for Identifying Heart and Liver Specific Toxicity of Antiviral Drugs.**
James McKim, Ph.D., DABT
CeeTox Inc.

2:30 pm **3. The Importance and Role of Formulation in Antiviral Drug Development Programs.**
Anthony Ham, Ph.D.
ImQuest BioSciences

3:00 pm **4. Regulatory Milestones and Requirements in Antiviral Drug Development.**
Martine Kraus, Ph.D.
Tobira Therapeutics

3:30 pm **5. Panel Discussion.**
Robert Buckheit, Ph.D.
ImQuest BioSciences

COFFEE BREAK

MARKET STREET FOYER

3:45 pm – 4:15 pm

OPENING GREETINGS AND WELCOME TO SAN FRANCISCO

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

GRAND BALLROOM A

4:15 pm – 4:30 pm

4:15 pm **6. Welcome Address.**
Philip Furman, Ph.D.
Furman Biotech Consulting

KEYNOTE ADDRESS

Chair(s): Robert Buckheit, Ph.D.

GRAND BALLROOM BC

4:30 pm – 5:30 pm

4:30 pm **7. Know Thine Enemy: Using Virology and Immunology to Develop a Multifaceted Approach to Dengue Antivirals.**
Eva Harris, Ph.D.
University of California, Berkeley

OPENING RECEPTION

ATRIUM 2-5

5:30 pm – 7:30 pm

Sunday, May 12, 2013

CONTINENTAL BREAKFAST

MARKET STREET FOYER

7:15 am – 8:15 am

ORAL SESSION 1: MINI-SYMPOSIUM – LEGACY OF TONY HOLÝ: NUCLEOTIDES IN THE TREATMENT AND PREVENTION OF CHRONIC VIRAL INFECTIONS

Chair(s): Tomas Cihlar, Ph.D. and Graciela Andrei, Ph.D.

GRAND BALLROOM BC

8:15 am – 12:00 pm

8:15 am **8. A Personal Note on the Contributions and Legacy of Antonín Holý.**
Erik De Clercq, Ph.D.
Rega Institute

8:30 am **9. A Tribute to Antonín Holý.**
John Martin, Ph.D.
Gilead Sciences

9:00 am **10. Tenofovir in the Treatment of HIV Infection.**
Robert Schooley, M.D.
University of California, San Diego

9:30 am **11. Tenofovir in the Prevention of HIV Infection.**
Robert Grant, Ph.D.
University of California, San Francisco

COFFEE BREAK

MARKET STREET FOYER

10:00 am – 10:30 am

10:30 am **12. Nucleotide Analogs in Chronic Hepatitis B – from Hope to Reality.**
Henry Chan, Ph.D.
Hong Kong University

11:00 am **13. Nucleotides in the Treatment and Prophylaxis of Herpes and Other DNA Virus Infection.**
Richard Whitley, M.D.
University of Alabama at Birmingham

11:30 am **14. Future Potential and Therapeutic Opportunities for Nucleoside Phosphonates.**
Tomas Cihlar, Ph.D.
Gilead Sciences

GERTRUDE ELION AWARD LECTURE

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

GRAND BALLROOM BC

1:30 pm – 2:15 pm

1:30 pm **15. My Antiviral Research in Fukushima, Leuven and Kagoshima.**
Masanori Baba, M.D., Ph.D.
Kagoshima University

ORAL SESSION 2: HEPATITIS AND HIV

Chair(s): William Delaney, Ph.D. and Dirk Daelemans, Ph.D.

GRAND BALLROOM BC

2:15 pm – 4:30 pm

2:15 pm **16. Discovery of 6-(Indol-2-yl)Pyridine-3-Sulfonamides as Novel HCV Inhibitors Targeting the Viral NS4B.**
Gary M. Karp, Xiaoyan Zhang, Nanjing Zhang, Guangming Chen, Anthony Turpoff, Neil Almstead, Zhengxian Gu, Joseph Colacino
PTC Therapeutics, South Plainfield, NJ, United States

2:30 pm **17. Next-Generation HCV NS5A Inhibitor: *In Vitro* Antiviral Optimization for Pan-Genotypic Activity and Preclinical Profile.**
Cyril B. Dousson[1], Christophe C. Parsy[1], David D. Dukhan[1], Jean-L. Paparin[1], Francois-R. Alexandre[1], Maria Seifer[2], Xin-R. Pan-Zhou[2], David N. Standring[2]
[1]Idenix Pharmaceuticals, Montpellier , France, [2]Idenix Pharmaceuticals, Cambridge, MA, United States

COFFEE BREAK

MARKET STREET FOYER

2:45 pm – 3:15 pm

3:15 pm **18. Identification of PT725: a Potent, Selective and Orally Bioavailable Small Molecule That Targets the Hepatitis C Virus NS4B Protein.**
Jason D. Graci, Zhengxian Gu, Stephen P. Jung, Gary Karp, Neil G. Almstead, Joseph M. Colacino
PTC Therapeutics, Inc., South Plainfield, NJ, United States

3:30 pm

19. Flexible Nucleotides as Antivirals.

H. Peters[1], H. Senderowitz[2], K. Seley-Radtke[1]
[1]University of Maryland Baltimore County, Baltimore, MD, United States, [2]Bar-Ilan University, Ramat-Gan , Israel

3:45 pm

20. Replication Inhibition by Small-Molecules Targeting the HIV Rev-Crm1 Interaction.

Eline Boons[1], Thomas Vercruysse[1], Sharon Shacham[2], Yosef Landesman[2], Erkan Baloglu[2], Sharon Tamir[2], Christophe Pannecouque[1], Dirk Daelemans[1]
[1]Rega Institute, KU Leuven, Leuven, Belgium, [2]Karyopharm Therapeutics, Natick, MA, United States

4:00 pm

21. Evolution of Pyrimidinedione HIV Therapeutic Agents with Improved Solubility and Metabolic Stability.

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

4:15 pm

22. A Novel Class of Antivirals for HIV/AIDS Intervention Revealed by Targeting the HIV Vif Protein Dimerization Domain.

Harold C Smith[1,2], Ryan P Bennett[2]
[1]Dept. Biochemistry University of Rochester, Rochester, NY, United States, [2]OyaGen, Inc, Rochester, NY, United States

POSTER SESSION 1: RETROVIRUSES, HEPATITIS VIRUSES, RESPIRATORY VIRUSES, AND ANTIVIRAL METHODS

GRAND BALLROOM A

4:30 pm – 6:30 pm

23. The Effect of Lopinavir / Ritonavir an Antiretroviral Drug on the Antimalarial Activity of Artemether or Artemether / Lumefantrine in a Mouse Model of Plasmodium Berghei.

Oyindamola O. Abiodun[1], John A. Akinbo[2], Olushola D. Ojurongbe[2]
[1]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo Statte, Nigeria, [2]Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Oshogbo, Oshogbo, Oyo State, Nigeria,

24. Compounds Synthesized with a Novel Scaffold of (1,5)-Di-Substituted Non-Nucleosidic Uracil Inhibit Foci Formation by Hepatitis C Virus (HCV).

Abdullah A. Awadh[1], Helen S. Gureeva[2], Mikhail S. Novikov[2], Luis M. Schang[1]
[1]Departments of Biochemistry and of Medical Microbiology and Immunology and Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada, [2]Volgograd Medical Scientific Center, Volgograd, Volgograd Oblast, Russia

25. Computer-Aided Discovery and Synthesis of Novel Anti-HCV Compounds.

Marcella Bassetto[1], Pieter Leyssen[2], Johan Neyts[2], Andrea Brancale[1]
[1]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff, United Kingdom, [2]Rega Institute for Medical Research, Leuven, Belgium

26. Small Molecule Agonists of the Rig-I Pathway and Their Broad Spectrum Inhibition of Hepatitis Viruses; Including Hepatitis C and Hepatitis B Virus.

Kristin Bedard[1], Myra Wang[1], Kerry Fowler[1], Wazir Abdullahi[1], Michael Gale, Jr.[2], Shawn Iadonato[1]
[1]KINETA, Inc., Seattle, WA, United States, [2]University of Washington, Seattle, WA, United States

27. Anti-HIV Metal Complexes of Pyridine-Fused Macrocyclic Polyamines Targeting the Cellular HIV Co-Receptors CXCR4 and CCR5.

Thomas W. Bell[1], Sunil Hamal[1], Dana Huskens[2], Thomas D'huys[2], Dominique Schols[2]
[1]Department of Chemistry, University of Nevada, Reno, NV, United States, [2]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

28. Synthesis and Evaluation of New Hepatitis C Virus NS3 Protease Inhibitors with an Azetidine Ring at the P2 Position.

Lavanya Bondada[1], Franck Amblard[1], Jerome Courcambeck[3], Gilles Roche[3], Tamara McBrayer[2], Philippe Halfon[3], Steven Coats[2], Raymond Schinazi[1]
[1]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, Decatur, GA, United States, [2]RFS Pharma, LLC, Tucker, GA, United States, [3]Genoscience-Pharma, Marseille, France

29. Hit-To-Lead Optimization in a Series of Tetrahydroquinolines for the Treatment of HCV.

Eda Canales, Roland Saito, Philip Morganelli, Robin Higgins, Rudolf Beran, Caroline Bush, Matthew Paulson, Scott Lazerwith
Gilead Sciences, Foster City, CA, United States

30. Phenothiazines Inhibit HCV Entry by Increasing the Fluidity of Cholesterol-Rich Membranes.

Zhilei Chen[1], Ana Chamoun-Emanuelli[1], Eve-Isabelle Pecheur[2], Simeon Rudo[1]
[1]Texas A&M University, College Station, TX, United States, [2]Universite de Lyon, Lyon, France

31. Anti-Adhesive Properties of Plant Extracts Cystus052 and Ladania067 as a Broad Range Antiviral Mechanism Against Respiratory Viral Pathogens.

Ehrhardt Christina[1], Haasbach Emanuel[2], Lapuse Julia[3], Hrincius Eike-Roman[1,4], Planz Oliver[2], Stephan Ludwig[1]
[1]Institute of Molecular Virology (IMV), University of Muenster, Germany, [2]Interfaculty Institute of Cell Biology, Department of Immunology, University of Tuebingen, , Germany, [3]Teutopharma GmbH, Glandorf, , Germany, [4]St. Jude Children's Research Hospital, Memphis, TN, United States

32. Novel HCV Binding, Fusion and Envelope Fluidity Assays Identify the Antiviral Mechanisms of the Natural Products Epigallocatechin Gallate and Curcumin.

Che C. Colpitts[1], Angga Kusuma[2], Eike Steinmann[2], Luis M. Schang[1]
[1]University of Alberta, Edmonton, Canada, [2]Twincore, Hannover, Germany

33. Rapid and Convenient Assays to Assess Potential Inhibitory Activity on *In Vitro* Hepatitis A Replication.

Yannick Debing[1], Gerardo G. Kaplan[2], Johan Neyts[1], Dirk Jochmans[1]
[1]University of Leuven, Leuven, Belgium, [2]Food and Drug Administration, Bethesda, MD, United States

34. Interferon Alpha and Ribavirin are Potent Inhibitors of Hepatitis E Virus Replication *In Vitro*.

Yannick Debing, Kai Dallmeier, Johan Neyts
University of Leuven, Leuven, Belgium

35. Novel Norbornane-Based Nucleoside and Nucleotide Analogues and Their Antiviral Activities.

Milan Dejmek[1], Michal Šála[1], Hubert Hřebabecký[1], Graciela Andrei[2], Jan Balzarini[2], Lieve Naesens[2], Johan Neyts[2], Radim Nencka[1]

[1]Gilead Sciences & IOCB Research Centre, Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic, [2]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

36. Alpharetroviral Vectors are Suitable Tools for Tre-Recombinase Based HIV-1 Gene Therapy.

Danilo Dubrau[1], Helga Hofmann-Sieber[1], Jan Chemnitz[1], Ilona Hauber[1], Axel Schambach[2], Christopher Baum[2], Frank Buchholz[3], Joachim Hauber[1]

[1]Heinrich Pette Institute – Leibniz Institute for Experimental Virology, Hamburg, Germany, [2]Hannover Medical School, Hannover, Germany, [3]University of Technology Dresden, Germany

37. HIV-Specific Promoters to Eliminate HIV-Infected Cells by Gene Therapy.

Nejat Düzgüneş, Senait Gebremedhin, Amy Au, Matthew Milnes, Krystyna Konopka

Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA, United States

38. Reduce the Risk in Vulnerability in HIV.

Philip Enyan

University of Ghana, Accra, Greater Accra, Ghana

39. Can Small Molecule Inhibition of the HIV Gp41 603–609 Dicysteine Loop Inhibit Viral Replication and Reverse the HIV-Induced Elevation of Camp and Il-10?

William Estrin[1], Joseph Long[2], Tiffany Jauhari[2]

[1]Saint Francis Memorial Hospital, San Francisco, CA, United States, [2]University of California, Santa Cruz B.S., Santa Cruz, CA, United States

40. From the Discovery of New Inhibitors of Rhinovirus Replication Toward the Development of an Antiviral Against a Wide Range of Enteroviruses.

Nisrine Falah[1], Sébastien Violot[2], Bruno Lina[1,3], Béatrice Riteau[4], Jean-Claude Cortay[1]

[1]VirPath, Université de Lyon, Lyon, France, [2]UMR 5086, Université de Lyon, France, [3]Laboratoire de Virologie, Hospices Civils de Lyon, Bron, France, [4]INRA, Tours, France,

41. An Innovative Approach for Multiplexed RSV Replicon Assay.

Jun Fan, Qin Yu

AstraZeneca, R&D Boston, Waltham, MA, United States

42. CYP24A1 Inhibitors and Vitamin D: A New Potential Anti-HCV Strategy?

Salvatore Ferla[1], Andrea Brancale[1], Hector DeLuca[2], Jinge G. Zue[2], Claire Simons[1]

[1]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff, United Kingdom, [2]Department of Biochemistry University of Wisconsin-Madison, Madison WI, United States

43. Antiviral Activity of Extracts from Cistus Incanus and Ribes Nigrum Against Different Influenza Virus Strains.

Emanuel Haasbach[1], Carmen Hartmayer[1], Julia Lapuse[2], Stephan Ludwig[3], Oliver Planz[1]

[1]University of Tuebingen, Tuebingen, Germany, [2]Teutopharma GmbH, Glandorf, Germany, [3]University of Muenster, Muenster, Germany

44. In Vitro Evaluation of the HIV Therapeutic IQP-0410 Through Transdermal Patch Delivery.

Anthony S. Ham, William P. Lustig, Ashlee D. Boczar, Karen M. Buckheit, Robert W. Buckheit Jr.
ImQuest BioSciences, Frederick, MD, United States

45. Development of Influenza Virus Inhibitors with a Higher Genetic Barrier to Resistance.

Tracy L. Hartman, Robert W. Buckheit, Jr.
ImQuest BioSciences, Frederick, MD, United States

46. Identification of a Novel Inhibitor of the HIV-1 Integrase-LEDGF Interaction.

Raymond Hewer[1], Maria A. Papathanasopoulos[2], Frederik H. Kriel[1], Angela Harrison[1,2], Salerwe Mosebi[1]
[1]Biomed, Mintek, Johannesburg, Gauteng, South Africa, [2]University of the Witwatersrand Medical School, Johannesburg, Gauteng, South Africa

47. Influenza A/NWS/33 (H1N1) Is Uniquely Sensitive to Oseltamivir Treatment in Mice Compared to More Recent Influenza Isolates.

Brett L. Hurst[1,2], Ramona Skirpstunas[1,4], Min-Hui Wong[1,2], Kerry Rood[1], Donald F. Smee[1,2], E. Bart Tarbet[1,2,3]
[1]Utah State University, Logan, UT, United States, [2]Institute for Antiviral Research, Logan, UT, United States, [3]School of Veterinary Medicine, Logan, UT, United States, [4]Department of Agriculture and Food, Logan, UT, United States

48. 1,2,4-Triazoles as Dual-Function Inhibitors of HIV-1 Reverse Transcriptase Polymerase and Ribonuclease H Activities.

Tatiana Ilina, Alexander Van Ry, Eva Nagy, Michael A. Parniak
University of Pittsburgh School of Medicine, Pittsburgh, PA, United States

49. HCV Polymerase Elongation Complex Formation and Characterization of a New Activity: NTP Mediated Nucleotide Excision.

Zhinan Jin[1], Vincent Leveque[1], Han Ma[1], Kenneth Johnson[2], Klaus Klumpp[1]
[1]Hoffman-La Roche Inc., Nutley, NJ, United States, [2]University of Texas at Austin, Austin, TX, United States

50. Cocktails of Formulated Short Synthetic shRNAs Show Potent Inhibition of Hepatitis C Virus in HCV-Infected Chimeric Mice.

Brian H. Johnston[1], Anne Dallas[1], Han Ma[2], Heini Ilves[1], Mark Behlke[3], Ian MacLachlan[4], Richard Harbottle[5], Klaus Klumpp[2]
[1]Somagenics, Inc., Santa Cruz, CA, United States, [2]Roche, Nutley, NJ, United States, [3]IDT, Coralville, IA, United States, [4]Tekmire Pharmaceuticals, Burnaby, BC, Canada, [5]Imperial College, London, London, United Kingdom

51. Non-Competitive Inhibition of Hepatitis B Virus Reverse Transcriptase Protein Priming and DNA Synthesis by Clevudine-Triphosphate.

Scott Jones[1], Eisuke Murakami[2], William Delaney[2], Phillip Furman[3], Jianming Hu[1]
[1]Penn State College of Medicine, Hershey, PA, United States, [2]Gilead Sciences, Foster City, CA, United States, [3]Pharmasset, Inc, Princeton, NJ, United States

52. Discovery and SAR Optimization of N-(Hetero)Aryl-6-(Indol-2-yl)Pyridine-3-Sulfonamides: PTC725, a Potent, Selective and Orally Bioavailable Development Candidate Targeting HCV NS4B.

Gary M. Karp, Xiaoyan Zhang, Nanjing Zhang, Anthony Turpoff, Guangming Chen, Neil Almstead, Zhengxian Gu, Joseph Colacino
PTC Therapeutics, South Plainfield, NJ, United States

53. Chemical Optimization of a Novel Class of 6-(Indol-2-yl)Pyridine-3-Sulfonamides Targeting HCV NS4B: Potent and Orally Bioavailable Compounds with an Improved ADMET Profile.

Gary M. Karp, Xiaoyan Zhang, Nanjing Zhang, Anthony Turpoff, Christie Morrill, Neil Almstead, Zhengxian Gu, Joseph Colacino
PTC Therapeutics, South Plainfield, NJ, United States

54. Antiviral Activity of Benzimidazol Derivatives Against Influenza A Virus.

Liubov A. Karpinskaia[1], Vladimir V. Zarubaev[1], Oleg I. Kiselev[1], Anatoly S. Morkovnick[2], Liudmila N. Divaeva[2]
[1]Influenza Research Institute, St. Petersburg, Russia, [2]Southern Federal University, Rostov-on-Don, Russia

55. Evaluation of Candidate Topical Microbicides in Pharmacokinetic and Pharmacodynamic *In Vitro* Models to Predict the Necessary Concentration Required to Prevent HIV Infection.

Mansoora Khaliq[1], Karen Buckheit[1], Charlene Dezutti[2, 3], Robert Buckheit Jr[1]
[1]ImQuest BioSciences, Frederick, MD, United States, [2]Magee Women's Research Institute, Pittsburg, PA, United States, [3]University of Pittsburg, Pittsburg, PA, United States

56. Amantadine Analogs That Inhibit MDCK Cell Infection by H1N1 2009 Influenza A Containing M2(S31N).

Antonios Kolocouris[1], Brent Johnson[2], Christina Tzitzoglaki[1], Francesc X. Sureda[4], Trevor Anderson[2], Anna K. Wright[3], Timothy A. Cross[3], David D. Busath[2]
[1]National and Kapodistrian Univ. of Athens, Athens, Attica, Greece, [2]Brigham Young Univ., Provo, UT, United States, [3]Florida State Univ., Tallahassee, FL, United States, [4]Univ. Rovira i Virgili, Reus, Tarragona, Spain

57. Evaluation of RNA-Knockdown Strategies for Modulation of Influenza Virus Matrix Gene Activity in Mammalian Cell Line.

Binod Kumar[1], Roopali Rajput[1], Latika Saxena[1], Mradul K. Daga[2], Madhu Khanna[1]
[1]Department of Respiratory Virology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India, [2]Department of Medicine, Maulana Azad Medical College, Delhi, India

58. QSAR Modeling of Anti-Influenza (A/H3N2) Activity.

Victor Kuz'min[1], Eugene Muratov[1], Anatoly Artemenko[1], Ekaterina Varlamova[1], Victor Lozitsky[2], Alla Fedchuk[2], Denis Fourches[3], Alexander Tropsha[3]
[1]A.V.Bogatsky Physical-Chemical Institute NAS of Ukraine, Odessa, Ukrenia, [2]I.I. Mechnikov Ukrainian Anti-Plague Research Institute, Odessa, Ukrenia, [3]University of North Carolina, Chapel Hill, NC, United States

59. A Michael-Acceptor-Type 3C Protease Inhibitor with Broad-Spectrum Anti-Rhinoviral Activity.

Céline Lacroix[1], Shyla George[2], Pieter Leyssen[1], Rolf Hilgenfeld[2], Johan Neyts[1]
[1]Laboratory for Virology and Experimental Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Lübeck, Germany

60. T-Cell Mediated Immunity Activation in HIV/HCV Coinfected Patients with AIDS.

NatalliaV. Matsiyeuskaya
Grodno State Medical University, Grodno, Grodno, Belarus

61. Differential Time-Dependent Activities of Small Molecule Inhibitors of Respiratory Syncytial Virus (RSV) Targeting Distinct Entry and Post-Entry Steps in the Virus Replication Cycle.

Krista McCutcheon, Michel Perron, Kirsten Stray, Robert Jordan, Tomas Cihlar
Gilead Sciences, Foster City, CA, United States

62. Mechanisms of Hypersusceptibility to EFDA-TP by NRTI- and NNRTI-Resistant HIV RTs.

Eleftherios Michailidis[1], Emily Ryan[1], Atsuko Hachiya[1, 2], Karen Kirby[1], Eiichi Kodama[3], Hiroaki Mitsuya[4, 5], Michael Parniak[6], Stefan Sarafianos[1]
[1]University of Missouri, Columbia, MO, United States, [2]AIDS Clinical Center, Tokyo, Japan, [3]Tohoku University, Miyagi, Japan, [4]NIH, Bethesda, MD, United States, [5]Kumamoto University, Kumamoto, Japan, [6]University of Pittsburgh, Pittsburgh, PA, United States

63. Ten Years Experience Observing Generation and Preservation of Specific Antibodies Against Measles in Children Born from HIV Positive Mothers with Confirmed HIV Infection.

Elena P. Nacharova, Olga V. Golaeva, Susanna M. Kharit
Research Institute of Children's Infections of the Federal Medical and Biological Agency, St. Petersburg, Russia, Russia

64. Activation of the Antiviral Agent T-705 (Favipiravir) by Hypoxanthine Guanine Phosphoribosyltransferase.

Lieve Naesens[1], Luke Guddat[2], Dianne Keough[2], André B.P. van Kuilenburg[3], Judith Meijer[3], Johan Vande Voorde[1], Jan Balzarini[2]
[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia, [3]Laboratory Genetic Metabolic Diseases, Academic Medical Center, Amsterdam, Netherlands

65. Galectin-3 Promotes HIV-1 Expression in Latently Infected Cells Through NF-κB Activation.

Mika Okamoto, Akemi Hidaka, Takayuki Hamasaki, Masaaki Toyama, Masanori Baba
Kagoshima University , Kagoshima, Japan

66. Surveillance for Neuraminidase Inhibitor Susceptibility of Influenza Viruses in 2011–2012: Application of New WHO Criteria.

Margaret Okomo-Adhiambo[1], Katrina Sleeman[1], Kristina Ballenger[2], Ha T. Nguyen[2], Anwar Abd Elal[2], Vasiliy P. Mishin[1], Larisa V. Gubareva[1]
[1]Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Battelle, Atlanta, GA, United States

67. Simultaneous Detection of Dengue Virus NS1 Antigen, IgM and IgG Antibodies in Febrile Cases in Ibadan, Nigeria.

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

68. Spirit: Switching to Rilpivirine/Emtricitabine/Tenofovir DF Single-Tablet Regimen from Boosted Protease Inhibitor Maintains HIV Suppression at Week 48.

Frank Pallela[1], Martin Fisher[2], Pablo Tebas[3], David Shamblaw[4], Hui Wang[5], Danielle Porter[5], Shampa De-Oertel[5], Damian McColl[5]
[1]Northwestern University, Evanston, IL, United States, [2]Brighton and Sussex University Hospital , Brighton , Sussex, United Kingdom, [3]University of Pennsylvania, Philadelphia, PA, United States, [4]La Playa Medical Group and Clinical Research, San Diego, CA, United States, [5]Gilead Sciences, Foster City, CA, United States

69. *In Vitro* Efficacy Profiling of Protease Inhibitors in Genotype 4A HCV Replicons.

Betty Peng, Katie Chan, Hadas Dvory-Sobol, Mei Yu, Angela Worth, Xiaowu Chen, William Delaney, Guofeng Cheng
Gilead Sciences, Foster City, CA, United States

70. Targeting the Organic Anion Transporter-3 (OAT3) with Probenecid as a Novel Anti-Influenza A Virus Strategy.

Olivia Perwitasari, Xiuzhen Yan, Scott Johnson, Ralph A. Tripp
Department of Infectious Diseases, University of Georgia, Athens, GA, United States

71. KPT-335, a Novel Inhibitor of Nuclear Export (SINE), Reduces Influenza A Virus Replication *In Vitro* and *In Vivo*.

Olivia Perwitasari[1], Scott Johnson[1], Sharon Shacham[2], Dilara McCauley[2], Sharon Tamir[2], Ralph A. Tripp[1]
[1]Department of Infectious Diseases, University of Georgia, Athens, GA, United States, [2]Karyopharm Therapeutics, Natick, MA, United States

72. The Discovery and Characterization of Novel Bioactive Small Molecules Targeting the Priming Complex of HIV-1.

Steve Peterson, Richard Guenther, Winnell Newman, Michael Ossi, Daniel Sternbach
Trana Discovery Inc., Cary, NC, United States

73. Are Antiviral Drugs Against Influenza Targeting Cell Signaling Pathways Inherently Toxic?

Oliver Planz[1], Emanuel Haasbach[1], Carmen Hartmayer[1], Stephan Pleschka[2], Stephan Ludwig[3]
[1]University of Tuebingen, Tuebingen, Germany, [2]University of Giessen, Giessen, Germany, [3]University of Muenster, Muenster, Germany

74. Evaluating the Impact of Educational Interventions on Use of Highly Active Antiretroviral Therapy and Adherence Behavior in Indian Human Immunodeficiency Virus Positive Patients: Prospective Randomized Controlled Study.

Rajesh Radhakrishnan[1], Sudha Vidyasagar[2], Muralidhar D. Varma[2]
[1]Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India, [2]Department of Medicine, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

75. Distinct Inhibition of Nonstructural Gene Expression Potently Reduces the Propagation of Influenza A Virus *In Vivo*.

Roopali Rajput[1], Prashant Kumar[1], Binod Kumar[1], Sonal Sharma[2], Latika Saxena[1], Madhu Khanna[1]
[1]University of Delhi, Delhi, India, [2]University College of Medical Sciences, Delhi, India

76. Comparative *In Vitro* Antiviral Activity of Multiple Classes of Influenza Inhibitors in Madin-Darby Canine Kidney Cells (MDCKs) and Normal Human Bronchial Epithelial Cells (NHBEs).

Madeleine W. Rodriguez, Christopher C. Mello, Michael Clarke, Robert Jordan, Tomas Cihlar, Gabriel Birkus
Gilead Sciences, Foster City , CA, United States

77. Antiviral Assessment of Penultimate Methyl Alkyloxyalkyl Esters of Phosphonomethoxypropyl Nucleosides Against HIV-1 *In Vitro*.

Jacqueline Ruiz[1,3], Karen W. Buckheit[2], Robert W. Buckheit Jr.[2], Ashlee Boczar[2], Kathy A. Aldern[1], James R. Beadle[1], Karl Y. Hostetler[1]
[1]UCSD, La Jolla, CA, United States, [2]ImQuest Biosciences, Frederick, MD, United States, [3]National University, La Jolla, CA, United States

78. The Triethylbenzene Scaffold; In the Search for Lectin Mimetics: a Novel Strategy for Anti-HIV Therapy.

Ana San-Félix[1], Eva Rivero-Buceta[1], Elisa G. Doyagüez[1], Elena Casanova[1], Ernesto Quesada[1], María-José Camarasa[1], Jan Balzarini[2], María Jesús Pérez-Pérez[1]
[1]Instituto de Química Médica, Madrid, Spain, [2]Rega Institute for Medical Research, Leuven, Belgium

79. Inhibitory Potential of Azadirachta Indica Juss (Neem) Leaves on Influenza A Virus Replication.

Latika Saxena, Roopali Rajput, Binod Kumar, Madhu Khanna
VP Chest Institute, University of Delhi, Delhi, India

80. Synthesis of Novel CADA Analog Prodrugs and Ring-Size Variants Designed to Act as Anti-HIV Agents Via Down-Modulation of the CD4 Receptor.

Emily D. Scarbrough[1], Dominique Schols[2], Thomas W. Bell[1]
[1]Department of Chemistry, University of Nevada, Reno, NV, United States, [2]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

81. Studies On HIV Integrase/LEDGF Inhibitory Activity of Compounds Isolated from Ethanolic Extract of Morinda Citrifolia L Noni

Periyasamy Selvam[1], Paul Pandi[1], Nouri Neamati[2], Tino Sanchez[2]
[1]Nova College of Pharmacy and Research, Ibrahimpatnam, Vijayawada, Andrapradesh, India, [2]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, Los Angele, CA, United States

82. HIV RNase H Inhibitory Activity of Novel Heterocyclic Compounds.

Periyasamy Selvam[1], Chitta Suresh Kumar[2], B. Babajan[2], Enzo Tramontano[3]
[1] Nova College of Pharmacy and Research , Ibrahimpatnam, Vijayawada, Andrapradesh, India, [2]Dept of Biochemistry, S. K. University, Anantapur, Andrapradesh, India, [3]Department of Life and environmental Sciences,University of Cagliari, Cittadella Universitaria di Monserrato SS554, Monserrato (Cagliari), Italy

83. Design, Molecular Modeling and Synthesis Of Novel Isatine Derivatives as Inhibitors of HIV Integrase/LEDGF Protein-Protein Interaction.

Periyasamy Selvam[1], Nouri Neamati[2], Tino Sanchenz[2], Anand Kumar V Raichurkar[3]
[1] Nova College of Pharmacy and Research , Ibrahimpatnam, Vijayawada , Andra Pradesh, India, [2]Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy , Los Angele, CA, United States, [3]Medicinal Chemistry, Infection iMED, AstraZeneca India Pvt. Ltd., Bellary Road, Hebbal, Bangalore, Karnataka, India

84. Design and Synthesis Of Quinazolin-4(3H)-Ones as Novel Inhibitors of HIV Integrase/LEDGF.

Periyasamy Selvam[1], Guoping Hu[2], Yun Tang[2], Xi Li[2], Jin Huang[2]
[1]Nova College of Pharmacy and Research, Ibrahimpatnam, Vijayawada, Andrapradesh, India, [2] School of Pharmacy, East China University of Science and Technology, Shanghai , China

85. Screening Small Molecule Inhibitors of Influenza-Host Protein Interactions.

Suganya Selvarajah, Sean Broce, Iting Jaing, Ian Brown, Erica Williams, Nicholas DeYarman, Beverly Freeman, Vinod Asundi
Prosetta Antiviral Inc, San Francisco, CA, United States

86. Synthesis and Anti-HCV Activity of Pyrazolo[3,4-D]Pyrimidine Carbocyclic Nucleosides.

Ashoke Sharon[1], Mohan Kasula[1], Mohammed TA Salim[2], Tuniki Balaraju[1], Masanori Baba[2], Chandralata Bal[1]
[1]Birla Institute of Technology, Ranchi, Jharkhand, India, [2]Center for Chronic Viral Diseases, Kagoshima University, Kagoshima, Japan

87. Antiviral Activity of Usnic Acid Derivates Against Influenza A/Aichi/2/68 *In Vivo*.

Anna A. Shtro[1], Olga A. Luzina[2], Marina P. Polovinka[2], Dmitry N. Sokolov[2], Nina I. Komarova[2], Nariman F. Salakhutdinov[2], Oleg I. Kiselev[1], Vladimir v. Zarubaev[1]
[1]Influenza Research Institute, Saint-Petersburg, Russia, [2]Novosibirsk Institute of Organic Chemistry, Novosibirsk, Russia

88. Evaluation of Acrylamide Grafted Sago Starch for Controlled Delivery of Anti-HIV Drug.

Akhilesh Vikram Singh
Indian Institute of Science, Bengaluru, Karnataka, India

89. Small Molecule Allosteric Activation of APOBEC3G as a Prophylactic Against HIV Infection.

Harold C. Smith[1,2], Kimberly M. Prohaska[1,2], William M. McDougall[2]
[1]OyaGen, Inc, Rochester, NY, United States, [2]Dept. Biochemistry, University of Rochester, Rochester, NY, United States

90. Adding of Interferon-Gamma to Interferon-Alpha and Ribavirin Increase the Efficacy of Chronic Hepatitis C Patients Therapy.

Tamara V. Sologub[1], Igor V. Volchek[2]
[1]Research Institute of Influenza, St. Petersburg, Russia, [2]DiscoveryMed Ltd, St. Petersburg, Russia

91. A Cross-Sectional Analysis of Antiviral in the First Cohort of HIV-Infected Children in North Eastern Uganda.

Muhamad Ssenjala[1], Chris Ojakol[2], Getrude Nalutaya[3], Zubair Sempebwa[1]
[1]Mbarara University of Science & Technology, Mbarara, Uganda, [2]Busoga University, Iganga, Iganga Distrct, Uganda, [3]Ndejje University, Ndejje, Luwero Distrct, Uganda

92. Mutational Analysis of the Binding Pocket of Diketoacid L-742,001 in the Influenza PA Endonuclease.

A. Stevaert[1], R. Dallocchio[2], A. Dessì[2], N. Pala[3], D. Rogolino[4], M. Sechi[3], L. Naesens[1]
[1]Rega Institute, KU Leuven, Leuven, Belgium, [2]Istituto di Chimica Biomolecolare, Li Punti, CNR, Italy, [3]Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy, [4]Dipartimento di Chimica Gen. ed Inorganica, Università di Parma, Parma, Italy

93. Effects of Oseltamivir Treatment on Cytokine Production During an Influenza A/Ca/04/2009 (H1N1) Virus Infection in Mice.

E. Bart Tarbet, Brett L. Hurst, Miles K. Wandersee, Min-Hui Wong, Donald F. Smee
Utah State University, Logan, UT, United States

94. Inhibition of Hepatitis B Virus Replication by Analogs of HIV RNase H and Integrase Antagonists.

John Tavis[1], Marvin Meyers[1], Xiaohong Cheng[1], Yuan Hu[2]
[1]Saint Louis University, Saint Louis, MO, United States, [2]Chongqing Medical University, Chongqing, China

95. Development of a Robust RSV Replicon Assay for High-Throughput Screening.

Choi Lai Tiong-Yip[1], Helen Plant[2], Paul Sharpe[2], Kirsty Rich[2], Elise Gorseth[1], Qin Yu[1]
[1]Infection Innovative Medicines Unit, AstraZeneca R&D Boston, Waltham, MA, United States, [2]Discovery Sciences, AstraZeneca R&D Alderley Park, Macclesfield, Cheshire, United Kingdom

96. 2'-Fluoro-2'-Deoxypurineriboside Protides: a Step Forward Towards Developing Influenza Virus Polymerase Inhibitors.

Evelien Vanderlinden[1], Silvia Meneghesso[2], Andrea Brancale[2], Jan Balzarini[1], Christopher McGuigan[2], Lieve Naesens[1]
[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Welsh School of Pharmacy, Cardiff University, Cardiff, United Kingdom

97. Modification of CCL5/RANTES Hot Spots Delivers Potent Anti-HIV-1 Full-Length and Peptide Derivatives Acting as CCR5 Antagonists.

Luca Vangelista
San Raffaele Scientific Institute, Milan, Italy

98. Optimizing the Unsymmetrical Structure of Benzyl-Tailed CADA Analogs to Improve Their CD4 Down-Modulating and Anti-HIV Activity.

Kurt Vermeire[1], Reena Chawla[2], Victor Van Puyenbroeck[1], Dominique Schols[1], Thomas W. Bell[2]
[1]Rega Institute for Medical Research, Leuven, Belgium, [2]University of Nevada, Reno, NV, United States

99. New Approach to Personalized Mono- and Combination Therapy of Chronic Hepatitis C Patients.

Igor V. Volchek[1], Tamara V. Sologub[2], Andrei S. Petrov[1]
[1]DiscoveryMed Ltd, St. Petersburg, Russia, [2]Research Institute of Influenza, St. Petersburg, Russia

100. Thiol-Disulfide Ratio as Universal Biomarker for Screening Antiviral Drugs for Personalized Therapy.

Igor V. Volchek[1], Tamara V. Sologub[2], Andrei S. Petrov[1], Natalia S. Loginova[3]
[1]DiscoveryMed Ltd, St. Petersburg, Russia, [2]Research Institute of Influenza, St. Petersburg, Russia, [3]V. I. Kulakov Research Center of Obstetrics, Gynecology & Perinatology, Moscow, Russia

101. *In Vitro* Anti-Influenza Virus Activity of Extracts of Sida Cordifolia L.

Ashish Wadhwani[1], Viral Patel[1], Suichi Sakamoto[2], Kurokawa Masahiko[2], Vijayan Pottekad[1]
[1]Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ootacamund, Tamil Nadu, India, [2]Department of Clinical Pharmacology, Kyushu University of Health and Welfare, Nobeoka, Miyazaki, Japan

102. Synthesis of Novel 2-Cyano7-Deaza-8-Azapurine Derived Nucleosides and Their Activity Against HCV.

Philip Wainwright[1], Adrian Maddaford[1], Mike Simms[1], Xiurong Zhang[1], Neil Forrest[1], David C. Pryde[2], Scott C. Sutton[3], Mark S. Betson[1]
[1]Peakdale Molecular Ltd, Chapel-en-le-Frith, High Peak, United Kingdom, [2]Pfizer Neusentis, Cambridge, Cambs., United Kingdom, [3]Pfizer Global Research and Development, San Diego, CA, United States

103. Novel 2,4-Diaminopyrimidine Nucleoside Phosphonate Antiviral Prodrugs.

Melissa M. Williams[1], Boris A. Kashemirov[1], Marcela Krecmerova[2], Tomas Tichy[2], Mark N. Prichard[3], John C. Drach[4], John M. Hilfinger[5], Charles E. McKenna[1]
[1]Department of Chemistry, University of Southern California, Los Angeles, CA, United States, [2]Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Prague, Czech Republic, [3]Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL, United States, [4]School of Dentistry, University of Michigan, Ann Arbor, MI, United States, [5]TSRL, Inc., Ann Arbor, MI, United States

104. The Griffithsin Dimer Is Required for High Potency Inhibition of HIV-1.

Jie Xue[1], Bart Hoorelbeke[2], Ioannis Kagiampakis[1], Borries Demeler[3], Jan Balzarini[2], Patricia J LiWang[1]
[1]University of California Merced , Merced, CA, United States, [2]Rega Institute for Medical Research, KU Leuven, Belgium, [3]The University of Texas Health Science Center at San Antonio Dept. of Biochemistry, San Antonio, TX, United States

105. Human Milk Sialylated Galactosides Inhibited Enterovirus 71 and A(H1N1) 2009 Influenza Infection in Respiratory and Gastrointestinal Cell Lines.

Betsy Yang[1], Hau Chuang[2]
[1]University of North Carolina, Chapel Hill, NC, United States, [2]Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Kaohsiung, Taiwan

106. Hepatitis B Virus Replication Kinetics Patterns and Their Impact on Screening Anti-HBV Therapeutic Agents in HepG2.2.15 and AD38 Cell Lines.

Yong-Yuan Zhang, Robert W. Buckheit, Jr.
ImQuest BioSciences, Inc., Frederick, MD, United States

107. Human Serum Contains Homologous Protein(S) to Those Found in Duck Serum That Enhance Duck Hepatitis B Virus cccDNA Synthesis: Implications for the Treatment of HBV.

Yong-Yuan Zhang, Robert W. Buckheit, Jr.
ImQuest BioSciences, Inc., Frederick, MD, United States

108. Frgtm Mice with Humanized Livers as a Robust Animal Model for HBV Drug Discovery.

Qiong Zhou[1], Qian Chen[1], Qiugang Lei[1], Xiaoyu Zhao[1], Elizabeth M. Wilson[2], John Bial[2], Henry Lu[1], Xinsheng Chen[1]
[1]WuXi AppTec (Shanghai), WaiGaoQiao Free Trade Zone, Shanghai, China, [2]Yecuris Corporation, Tualatin, OR, United States

Monday, May 13, 2013

CONTINENTAL BREAKFAST

MARKET STREET FOYER

7:00 am – 8:00 am

WILLIAM PRUSOFF YOUNG INVESTIGATOR AWARD

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

GRAND BALLROOM BC

8:00 am – 8:45 am

109. From Irrational to Rational Antiviral Drug Design.
Andrea Brancale, Ph.D.
Cardiff University

ORAL SESSION 3: MINI-SYMPOSIUM – STRATEGIES AND TACTICS IN DRUG DESIGN

Chair(s): Paul Scola, Ph.D. and Zlatko Janeba, Ph.D.

GRAND BALLROOM BC

8:45 am – 11:45 am

8:45 am **110. A Thermodynamic Approach to the Development of Novel Antivirals.**
Ernesto Freire, Ph.D.
Johns Hopkins University

9:15 am **111. Molecular Recognition in Drug Design.**
Daniel Cheney, Ph.D.
Bristol-Myers Squibb

9:45 am **112. Discovery of a Novel Allosteric Inhibitor of the HCV NS3/4A Protein Using Fragment Screening and Structure-Based Design.**
Andrew Woodhead, Ph.D.
Astex Pharmaceuticals

COFFEE BREAK

MARKET STREET FOYER

10:15 am – 10:45 am

10:45 am **113. The Discovery of an Allosteric NS5B Replicase Inhibitor for the Treatment of Hepatitis C Virus Infection.**
John Kadow, Ph.D.
Bristol-Myers Squibb

11:15 am **114. Discovery of MK-5172, a Macrocyclic Hepatitis C Virus NS3/4A Protease Inhibitor.**
Nigel Liverton, Ph.D.
Merck & Co., Inc.

LUNCH ON YOUR OWN

ORAL SESSION 4: INFLUENZA AND RESPIRATORY INFECTIONS

Chair(s): Bruno Canard, Ph.D. and Dale Barnard, Ph.D.

GRAND BALLROOM BC

1:30 pm – 4:30 pm

1:30 pm **115. RNA Synthesis, Capping and Repair in (+)RNA Viruses: Novel Targets for Drug Design.**
Bruno Canard, Ph.D.
Université de la Méditerranée

2:00 pm **116. Structure and Inhibition of the Drug-Resistant Mutants of the M2 Ion Channel of Influenza A Virus.**
Jun Wang[1], Yibing Wu[1], Chunlong Ma[2], Robert Lamb[2], William DeGrado[1]
[1]UCSF, San Francisco, CA, United States, [2]Northwestern University, Evanston, IL, United States

2:15 pm **117. New Small Molecule Entry Inhibitors Targeting Hemagglutinin-Mediated Influenza A Virus Fusion.**
Arnab Basu[1], Michael Caffrey[2], Dale L. Barnard[3], Lijun Rong[2], Terry L. Bowlin[1]
[1]Microbiotix Inc, Worcester, MA, United States, [2]University of Illinois at Chicago, Chicago, IL, United States, [3]Institute for Antiviral Research, Utah State University, Logan, UT, United States

2:30 pm **118. Mechanism of Action of Favipiravir (T-705) Revealed by a Novel Biochemical Assay Using Recombinant Influenza A Virus Polymerase.**
Zhinan Jin[1], Vivek K. Rajwanshi[1], David B. Smith[1], Baek Kim[2], Julian A. Symons[1], Lawrence M. Blatt[1], Leonid Beigelman[1], Jerome Deval[1]
[1]Alios BioPharma, Inc., South San Francisco, CA, United States, [2]University of Rochester Medical Center, Rochester, NY, United States

2:45 pm **119. Gp1001, A Host Targeted Influenza Therapy.**
Dary Faulds[1], Dale Barnard[2], John Morrey[2], Jiing-Huey Lin[1], Hsiao-Lai Liu[1], Bart Tarbet[2], William Guilford[1]
[1]Gemmus Pharma Inc, San Francisco, CA, United States, [2]Utah State University, Logan, UT, United States

COFFEE BREAK

MARKET STREET FOYER

3:00 pm – 3:30 pm

3:30 pm **120. Identification of a Potent Fusion Inhibitor of Influenza A Virus.**
K.K. Lai, F. Yang, K.Y. Yuen, R.Y. Kao
Hong Kong University, Hong Kong SAR, China

3:45 pm **121. TSR-026 – an Oral Drug Therapy for Oseltamivir-Resistant Influenza Infections.**
John M. Hilfinger[1], Donald F. Smee[2], Dawn M. Reyna[1], Mindy A. Collins[1], Crystal A. Jurkiewicz[1], Elke Lipka[1]
[1]TSRL, Inc., Ann Arbor, MI, United States, [2]Utah State University, Logan, UT, United States

4:00 pm **122. A Ferret Model to Facilitate the Establishment of Laboratory Correlates for Clinically Relevant Oseltamivir Resistance.**
H Marjuki, AP Chesnokov, VP Mishin, K Sleeman, M Okomo-Adhiambo , AI Klimov, LV Gubareva
Influenza Division, NCIRD, CDC, Atlanta, GA, United States

4:15 pm

123. A Novel Class of Highly Potent Small Molecule Inhibitors of Entero/Rhinovirus Replication with an Excellent Safety and Pharmacokinetic Profile are Highly Effective Against Enterovirus Infections in Mice.

Hendrik Jan Thibaut[1], Sung-Hoon Ahn[2], Aloys Tijsma[1], Chong-Kyo Lee[2], Eric Verbeken[3], Young-Sik Jung[2], Johan Neyts[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Korea Research Institute of Chemical Technology, Daejeon, South Korea, [3]Division of Morphology and Molecular Pathology, KU Leuven, Leuven, Belgium

POSTER SESSION 2: HERPESVIRUSES, POXVIRUSES, ENTEROVIRUSES, EMERGING VIRUSES, OTHER ANTIVIRAL AGENTS AND MEDICINAL CHEMISTRY

GRAND BALLROOM A

4:30 pm – 6:30 pm

124. Nano-Effect of Clay Minerals on Human Papillomavirus-Warts.

Abdulrhem T. Al-Ghazal

University of Mosul, Mosul, Nenaveh, Iraq

125. Genotypic and Phenotypic Herpes Simplex Virus Type 2 (HSV-2) Dynamics of Drug-Resistant Mutations During Antiviral Therapy in an Hematopoietic Stem Cell Transplant (HSCT) Recipient.

Graciela Andrei[1], Florence Morfin[2], André Boibieux[2], Sophie Ducastelle[2], Sarah Gillemot[1], Ghislain Opdenakker[1], Robert Snoeck[1]

[1]Rega Institute for Medical Research, KU Leuven, Belgium, [2]Hospices Civils de Lyon, Lyon, France

126. A Skeletal Hybridization Approach to Generate Novel α-Pyrone Analogs as Anti-HSV Agents.

Chandralata Bal[1], Srinivas Karampuri[1], Paromita Bag[2], Debprasad Chattopadhyay[2], Ashoke Sharon[1]

[1]Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India, [2]ICMR Virus Unit, Beliaghata, Kolkata, West Bengal, India

127. Inhibition of SARS-CoV by Thiazolidin-4-Ones.

Dale L. Barnard[1], Yohichi Kumaki[1], Kevin Bailey[1], Donald F. Smee[1], John D. Morrey[1], Roman Lesyk[2]

[1]Utah State University, Logan, UT, United States, [2]Danylo Halytsky Lviv National Medical University, Lviv, Ukrenia

128. Defining the Required Tissue Concentration of an Antiviral Agent to Totally Suppress Infection, Replication, and Transmission.

Karen W. Buckheit, Caitlin Buchholz, Ashlee Boczar, Robert W. Buckheit, Jr.

ImQuest BioSciences, Inc., Frederick, MD, United States

129. The Imquest Success Drug Development Platform: Enhancing Successful Drug Development Opportunities.

Robert W. Buckheit, Jr., Karen W. Buckheit, Christian Furlan-Freguia, Anthony Ham, Tracy L Hartman, Mansoora Khaliq, Todd B Parsley, Yong-Yuan Zhang

ImQuest BioSciences, Inc., Frederick, MD, United States

130. X-Ray Studies on the Mechanism of Inhibition of Foot-And-Mouth Disease Virus VPg-1 by FUTP.

María-José Camarasa[1], Gloria Fernández-Cureses[1], Sonia De Castro[1], Cristina Ferrer-Orta[2], Nuria Verdaguer[2], Esteban Domingo[3]
[1]Instituto de Química Médica (IQM-CSIC), Madrid, Spain, [2]Institut de Biología Molecular (IBMB-CSIC), Barcelona, Spain, [3]Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC), Madrid, Spain

131. Targeting CVB3 3A Protein: a Virtual Screening Approach.

Michela Cancellieri[1], Pieter Leyssen[2], Rachel Ulferts[3], Frank van Kuppeveld[3], Johan Neyts[2], Andrea Brancale[1]
[1]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, United Kingdom, [2]Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium, [3]Department of Infectious Diseases & Immunology, Utrecht University, Utrecht, Netherlands

132. Efficacy of N-Methanocarbathymidine (N-MCT) Against Herpes Simplex Virus Type 2 in a Genital Guinea Pig Model.

Rhonda Cardin[1], Fernando Bravo[1], Julie Earwood[1], Jennifer Clark[1], Derek Pullum[1], Robert Glazer[2], Aquilur Rahman[3], David Bernstein[1]
[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [2]Georgetown University, Washington, D.C., United States, [3]N & N Scientific, Inc., Rockville, MD, United States

133. Design, Synthesis and Evaluation of Potent Trisialic Acid (TSA) Compounds as Inhibitors of Ocular Adenovirus Type 37.

Naresh Chandra[1,2]
[1]Molecular Infection Medicine Sweden (MIMS), EMBL, Umeå, Västerbotten, Sweden, [2]Division of Virology, Department of Clinical Microbiology, Umeå University, Umeå, Västerbotten, Sweden

134. RNA Competition Screening Assays in Dengue Target-Based Drug Discovery.

Alex Chao, Christian Noble, Siew Pheng Lim, Pei Yong Shi
Novartis Institute for Tropical Diseases, Singapore, Singapore

135. The Highly Selective Inhibition of Epstein-Barr Virus Replication by Kay-02-41 Is Dependent on the Virus Thymidine Kinase.

Natacha Coen[1], Sophie Duraffour[1], Kazuhiro Haraguchi[2], Kaori Yamada[2], Joost J. Van den Oors[3], Jan Balzarini[1], Robert Snoeck[1], Graciela Andrei[1]
[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]School of Pharmacy, Showa University, Shinagawa, Japan, [3]Pathology Department, UZ Leuven, Leuven, Belgium

136. Altered Cyclic HPMPC Metabolism in Induced P3HR-1 Cells Accounts for Its Reduced Antiviral Activity Against Epstein-Barr Virus.

Natacha Coen, Sophie Duraffour, Lieve Naesens, Robert Snoeck, Graciela Andrei
Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

137. High Resolution Iodixanol Gradients Reveal Aberrant Capsid Formation Upon Small Molecule Blockade of Host Factor Targets Involved in Capsid Assembly.

Kiel Copeland, Hong Shi, Anatoliy Kitaygorodskyy, Connie Ewald, Caleb Declouette, Alfredo Calayag, Yemi akintunde, Clarence R Hurt
Prosetta Antiviral Inc, San Francisco, CA, United States

138. L-BHDU Requires VZV TK and Prevents Virus Replication by Competition with the Pyrimidine Biosynthesis Pathways.

Chandrav De[1], Uma S Singh[2], Chung K Chu[2], Jennifer F Moffat[1]
[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]University of Georgia, Athens, GA, United States

139. Repopulation of Ganciclovir-Resistant Cytomegalovirus by Wild Type Virus.

William L. Drew[1], Catherine Liu[1]
[1]*University of California, San Francisco, CA, United States,* [2]*University of California, San Francisco, CA, United States*

140. N-Alkyldeoxynojirimycin Derivatives with Novel Terminal Tertiary Amide Substitution Active Against Multiple Hemorrhagic Fever Viruses.

Yanming Du[1], Hong Ye[1], Tina Gill[2], Andrea Cuconati[1], Ju-Tao Guo[2], Timothy Block[1,2], Jinhong Chang[2], Xiaodong Xu[1]
[1]*Institute for Hepatitis and Virus Research, Doylestown, PA, United States,* [2]*Drexel University College of Medicine, Doylestown, PA, United States*

141. Mutations Associated with ST-246 Resistance are Not Found as Inter-Strain Polymorphisms Among a Total of 164 Orthopoxviruses.

Sophie Duraffour[1], Graciela Andrei[1], Gudrun Zöller[2], Annabel Rector[1], Dennis E Hruby[3], Doug Grosenbach[3], Robert Snoeck[1], Hermann Meyer[2]
[1]*Rega Institute for Medical Research, KU Leuven, Leuven, Belgium,* [2]*Bundeswehr Institute of Microbiology, Munich, Bavaria, Germany,* [3]*SIGA Inc., Corvallis, OR, United States*

142. ABCE1 Is a Host Factor Involved in Capsid Assembly for Multiple Viral Families.

Jean Francis[1], Marissa Baker-Wagner[1], Scott Long[1], Christine Nichols[1], Shao Feng Yu[1], Amethyst Chang[1], Dharma Prasad[2], Debendranath Dey[1]
[1]*Prosetta Antiviral Inc, San Francisco, CA, United States,* [2]*Prosetta Bioconformatics Private Limited, Mysore, Karnataka, India*

143. Castalagin as an Antiviral with Anti-Herpesvirus Potential.

Angel S. Galabov[1], Neli Vilhelmova[1], Stephane Quideau[2]
[1]*Institute of Microbiology, BAS, Sofia, Bulgaria,* [2]*Universite de Bordeaux, Pessac Cedex, France*

144. Cell-Free Protein Synthesis-Based Screens for Host-Targeted Antiviral Drug Discovery.

Emma KT Harrell[1], Xianfu Wu[2], James Chamberlin[1], Yoko Marwidi[1], Nu Lai Tsutsui[1], Eva Wong[1], Clarence R Hurt[1], Vishwanath R Lingappa[1]
[1]*Prosetta Antiviral Inc, San Francisco, CA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

145. A New and Five Known Antiviral Diterpenoids from the Leaf of Pinus Densiflora Against Human Papillomavirus *In Vitro*.

Yukyoung Jeon[1], Chi-Ung Moon[1], Hee-Jung Lee[2], Young Bong Kim[2], Song-Yi Ha[3], Ok Pyo Zee[3], Jong Hwan Kwak[3]
[1]*Gueulri R&D Center, Ansan, Gyeonggi-do, South Korea,* [2]*Konkuk University, Gwangjin-gu, Seoul, South Korea,* [3]*Sungkyunkwan University, Suwon, Gyeonggi-do, South Korea*

146. Comparison of Chikungunya Virus Isolates in Mice with Regard to Pathogenesis and Sensitivity to Effective Antiviral Treatment.

Justin Julander[1], Ashley Dagley[1], Jane Ennis[2], Jeff Turner[2]
[1]*Utah State University, Logan, UT, United States,* [2]*Defyrus Inc., Toronto, ON, Canada*

147. Isoflavone Agonists of IRF3-Dependent Signaling Elicit Potent Antiviral Activity Against Diverse Respiratory Viruses.

Shari M Kaiser[1], Kerry Fowler[1], Myra L Wang[1], Michael Gale, Jr.[2], Shawn P Iodonato[1], Kristin M Bedard[1]
[1]*KINETA, Inc., Seattle, WA, United States,* [2]*Departments of Immunology and Microbiology, University of Washington, Seattle, WA, United States*

148. Selective Antiviral Activity of a Novel Compound Class Developed in Silico Based on the Structure of the E Protein of Dengue Virus.

Suzanne Kaptein[1], Tine De Burghgraeve[1], Surender Singh Jadav[2], Venkatesan Jayaprakash[2], Pieter Leyssen[1], Johan Neyts[1]
[1]Rega Institute – KULeuven, Leuven, Belgium, [2]Birla Institute of Technology, Mesra, India

149. A High-Throughput Assay for Assessment of Antiviral Activity Against Human Norovirus Proteases.

Brent E. Korba[1], Prasanth Viswanathan[1], Jared May[1], Roger G. Ptak[2], Larry J. Ross[3], Lucile White[3]
[1]Georgetown University Medical Center, Dept. Microbiol. & Immunol., Washington, DC, United States, [2]Southern Research Institute, Department of Infectious Disease Research, Frederick, MD, United States, [3]Southern Research Institute, High Throughput Screening Center, Birmingham, AL, United States

150. New Prodrugs of Acyclic Nucleoside Analogues and Phosphonomethyl Derivatives.

Marcela Krečmerová[1], Jiří Blažek[1], Graciela Andrei[2], Jan Balzarini[2], Snoeck Robert[2]
[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague 6, Czech Republic, [2]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

151. Novel Tyrosine N-Alkyl Amide Prodrugs of (S)-HPMPC and (S)-HPMPA: Preliminary SAR Studies and *In Vitro* Antiviral Activities Against HCMV, Cowpox, and Vaccinia Viruses.

Ivan S. Krylov[1], Boris A. Kashemirov[1], John C. Drach[2], Mark N. Prichard[3], Mindy Collins[4], Dawn Reyna[4], John M. Hilfinger[4], Charles E. McKenna[1]
[1]Department of Chemistry, University of Southern California, Los Angeles, CA, United States, [2]School of Dentistry, University of Michigan, Ann Arbor, MI, United States, [3]Department of Pediatrics, University of Alabama School of Medicine, Birmingham, AL, United States, [4]TSRL Inc., Ann Arbor, MI, United States

152. Molecular Mechanism of Action of LPCRW_0005, a Benzonitrile Derivate, on the Replication of Human Rhinovirus 14.

Céline Lacroix[1], Manon Roche[2], Mathy Froeyen[3], Patrice Vanelle[2], Thierry Terme[2], Johan Neyts[1], Pieter Leyssen[1]
[1]Laboratory for Virology and Experimental Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Laboratory for Radical Pharmaco-Chemistry, Institute for Radical Chemistry ICR, UMR 7273, CNRS-Aix-Marseille Univ, Marseille, France, [3]Laboratory for Medicinal Chemistry, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

153. Molecular Dissection of the Timing and Mechanism of Action of a Small Molecule with Robust Activity Against Host Factors That Participate in Rabies Virus Capsid Assembly.

Usha F Lingappa[1], Xianfu Wu[2], Amanda Macieik[1], Kumar Paulvannan[1], Dennis Solas[1], Andy Atuegbu[1], Ken Tsutsui[1], Vishwanath R Lingappa[1]
[1]Prosetta Antiviral Inc, San Francisco, CA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States

154. Proteolysis Inhibitor Aminocaproic Acid Shows Both Antiviral and Antibacterial Activity.

Viktor Lozitsky, A. Fedchuk, T. Gridina, L. Mudrik, L. Shitikova, L. Socheslo
Ukrainian I.I. Mechnikov Anti-Plague Research Institute, Odessa, Ukrenia

155. The Benzamide-Based Compound K22 Exhibits Anti-Coronavirus 229E Potency by Targeting the Activity of Viral Nonstructural Protein 6.

Anna Lundin[1], Ronald Dijkman[2], Tomas Bergström[1], Nina Kann[3], Beata Adamiak[1], Charles Hannoun[1], Volker Thiel[2], Edward Trybala[1]

[1]Dept. of Clinical Virology, University of Gothenburg, Gothenburg, Sweden, [2]Institute of Immunobiology, Kanontal Hospital, St Gallen, Switzerland, [3]Dept.of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

156. Interactions of Acyclic Nucleoside Phosphonates with Selected Renal SLC and ABC Transporters.

Jana Mandikova[1], Marie Volkova[1], Petr Pavek[1], Michal Česnek[2], Zlatko Janeba[2], Frantisek Trejtnar[1]

[1]Charles University in Prague, Faculty of Pharmacy in Hradec Kralove, Hradec Kralove, Czech Republic, [2]Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Prague, Czech Republic

157. *In Vitro* Study of the Effect of Nitazoxanide on the Replication of Dengue Virus and Yellow Fever Virus.

Marcelo D. F. Meneses[1,3], Rafael S. Duarte[1,3], Edimilson R. Migowski[2,3], Davis F. Ferreira[1,3]

[1]Instituto de Microbiologia – Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Instituto de Pediatria – Federaql University of Rio de Janeiro, Rio de Janeiro, Brazil, [3]Farmoquimica, Rio de Janeiro, Brazil

158. Obscurum Per Obscurius: Computer-Aided Design of Novel Antivirals Using Simplex Approach.

Eugene Muratov[1], Victor Kuz'min[1], Ekaterina Varlamova[1], Anatoly Artemenko[1], Victor Lozitsky[2], Alla Fedchuk[2], Tatiana Gridina[2], Lubov Mudrik[2]

[1]A.V.Bogatsky Physical-Chemical Institute NAS of Ukraine, Odessa, Ukrenia, [2]I.I. Mechnikov Ukrainian Anti-Plague Research Institute, Odessa, Ukrenia

159. Conformational Flexibility of the Dengue Virus RNA-Dependent RNA Polymerase Revealed by a Complex with an Inhibitor.

Christian G. Noble, Siew Pheng Lim, Yen-Liang Chen, Pei-Yong Shi

Novartis Institute for Tropical Diseases, 10 Biopolis Road, 05-01 Chromos, Singapore

160. Anti-Poliovirus Compounds from Medicinal Plants Selected from the Nigerian Ethno-Medicine.

Omonike O. Ogbole [1], Johnson A. Adeniji[2], Edith O. Oriabure[1], Soup T.R Kamdem[3], Sajan Shyaula[4], Iqbal M. Choudary[4]

[1]Department of Pharmacognosy, Faculty of Pharmacy University of Ibadan, Ibadan, Oyo, Nigeria, [2]W.H.O Polio Laboratory, Department of Virology, College of Medicine, University of Ibadan., Ibadan, Oyo, Nigeria, [3]Department of Organic Chemistry, Higher Teachers' Training College, University of Yaounde I, Yaounde, Cameroon, [4]HEJ Research Institute of Chemistry, International Center for Chemistry and Biological Sciences, University of Karachi,, Karachi, Pakistan

161. Human Cytomegalovirus Resistance to Deoxyribosylindole Nucleosides Maps to a Point Mutation in the Terminase Subunit Encoded Gene UL89.

Quang Phan[1], Ellie D. Hall[2], Julie M. Breitenbach[3], Katherine Z. Borysko[3], Leory B. Townsend[4], Jeremy P. Kamil[2], John C. Drach[3], Brian G. Gentry[1]

[1]Drake University College of Pharmacy and Health Sciences, Des Moines, IA, United States, [2]Louisianna State University Department of Microbiology and Immunology, Shreveport, LA, United States, [3]University of Michigan Department of Biologic and Materials Sciences, Ann Arbor, MI, United States, [4]University of Michigan Department of Medicinal Chemistry, Ann Arbor, MI, United States

162. Cellular Factors Involved in Dengue-3 Virus Entry into the Host Cell.

Luana É. Piccini, Viviana Castilla, Elsa B. Damonte
Laboratory of Virology, School of Sciences, University of Buenos Aires- IQUIBICEN,CONICET, Buenos Aires, Argentina

163. *In Vitro* Antiviral Activity of β-Carbolines Against RNA Viruses.

Luana É. Piccini[1], Juan D. Panozzo Zénere[1], Elsa B. Damonte[1], María A. Ponce[2], Viviana Castilla[1]
[1]Laboratorio de Virología, Departamento de Química Biológica, IQUIBICEN/CONICET. Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina, [2]Departamento de Química Orgánica, CIHIDECAR-CONICET. Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina

164. Synergistic Efficacy of Mycophenolic Acid Or Mycophenolate-Mofetil with Acyclovir Against Herpes Simplex Virus Type 1 in Mouse Models.

Debra C. Quenelle, Deborah J. Collins, Terri L. Rice, Mark N. Prichard
University of Alabama School of Medicine, Birmingham, AL, United States

165. Clearance of a Human Norovirus Replicon from the Host Cell by the Protease Inhibitor Rupintrivir.

Joana Rocha-Pereira[1], MSJ Nascimento[1], Johan Neyts[2], Dirk Jochmans[2]
[1]L. Microbiologia, D. Ciências Biológicas, Faculdade de Farmácia and Centro de Química Medicinal, Universidade do Porto, Porto, Portugal, [2]Rega Institute for Medical Research, University of Leuven, Leuven, Belgium

166. Biochemical Characterization and Structural Analysis of NS2B/NS3 Protease from St. Louis Encephalitis Virus for Inhibitor Design.

Naoki Sakai[1,2], Lili Zhu[1,2], Steffen Pahlow[1,2], Subhash Vasudevan[3], Rolf Hilgenfeld[1,2,4]
[1]Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Ratzeburger Allee 160, 23538, Lübeck, Germany, [2]German Center for Infection Research (DZIF), University of Lübeck, Lübeck, Germany, [3]Duke – National University of Singapore Graduate Medical School, Singapore, Singapore, [4]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

167. Development of a Novel Animal Component Free Medium That Promotes the Growth of Various Animal Cell Lines for the Production of Viral Vaccines.

Rourou Samia, Majoul Semi, Trabelsi Khaled, and Héla kallel
Laboratory of Molecular Microbiology, Vaccinology and Biotechnology Development, Viral Vaccines Research & Development Unit, Tunis, le belvedère, Tunisia

168. Antiviral Activity of Favipiravir Against Experimental Rift Valley Fever Virus Infection.

Dionna M. Scharton[1,2], Kevin W. Bailey[1,2], Zachary Vest[1,2], Ramona Skirpstunas[4], Yousuke Furuta[5], Brian B. Gowen[1,2,3]
[1]Department of Animal, Dairy, and Veterinary Science, Logan, UT, United States, [2]Institute for Antiviral Research, Logan, UT, United States, [3]School of Veterinary Medicine, Utah State University, Logan, UT, United States, [4]Department of Agriculture and Food, State of Utah, Logan, UT, United States, [5]Toyama Chemical Company, Ltd., Tokyo, Japan

169. Inhibition of Chikungunya Virus Replication by T-705 (Favipiravir) and Identification of Resistance Associated Mutations in the RNA-Dependent RNA Polymerase.

N. Segura Guerrero [1], L. Delang[1], G. Querat[2], B. Martina[3], X. de Lamballerie [2], M. van Hemert [4], P. Leyssen[1], J. Neyts[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Université de la Méditerranée, Marseille, France, [3]Erasmus Medical Center, Rotterdam, Netherlands, [4]Leiden University Medical Center, Leiden, Netherlands

170. The ATP-Specific Priming Site of Dengue Virus Polymerase – a Target Site for the Development of Antivirals.

Barbara Selisko, Supanee Potisopon, Stéphane Priet, Cécilia Eydoux, Axelle Collet, Etienne Decroly, Jean-Claude Guillemot, Bruno Canard

Architecture et Fonction des Macromolécules Biologique (AFMB) – Antiviral Drug Discovery Platform (AD2P), Aix-Marseille University – CNRS, Marseille, France

171. Generation and Characterization of Neutralizing Human Recombinant Antibodies Against Antigenic Site Π of Rabies Virus Glycoprotein.

Lina Sun[1], Jingshuang Wei[2], Chuan Li[1], Qing Tang[1], Dexin Li[1], Mifang Liang[1]

[1]National Institute for Viral Disease Control and Prevention, China CDC, Beijing, Beijing, China, [2]2. New Drug R&D Center, State Key Laboratory of Development of Antibody Drugs, North China Pharmaceutical Corporation, Shijiazhuang, Hebei, China

172. Binding of Glutathione to Enterovirus Capsids Is Essential for Virion Morphogenesis and Depletion of Glutathione Results in an Antiviral Effect.

Hendrik Jan Thibaut[1], Lonneke Van der Linden*, Ping. Jiang[6]*, Bert Thys[3], Aniko Paul[6], Lole Canela[4], Bart Rombaut[3], Maria-Jesus Pérez Pérez[4], Eckard Wimmer[6], Frank Van Kuppeveld[2,5], Johan Neyts[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [3]Dept. Pharmaceutical Biotechnology & Molecular Biology, Vrije Universiteit Brussel, Brussels, Belgium, [4]Instituto de Química Médica, Madrid, Spain, [5]Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands, [6]Department of Molecular Genetics and Microbiology, Stony Brook University School of Medicine, New York, USA

**equal contribution*

173. A Novel Class of Highly Potent Small Molecule Inhibitors of Entero/Rhinovirus Replication That Target the Non Structural Protein 2C.

Hendrik Jan Thibaut[1], Chong-Kyo Lee[2], Lonneke Van der Linden[1,3], Bruno Coutard[4], Bruno Canard[4], Frank Van Kuppeveld[3,5], Young-Sik Jung[2], Johan Neyts[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Korea Research Institute of Chemical Technology, Daejeon, South Korea, [3]Radboud University Nijmegen Medical Centre , Nijmegen, Netherlands, [4]Aix-Marseille Université, Marseille, France, [5]Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands

174. Synthesis and Antiviral Activities of Hexadecyloxypropyl Prodrugs of Acyclic Nucleoside Phosphonates Containing Guanine Or Hypoxanthine and a (S)-HPMP Or PEE Acyclic Moiety.

Tomas Tichy[1], Graciela Andrei[2], Robert Snoeck[2], Jan Balzarini[2], Martin Dracinsky[1], Marcela Krecmerova[1]

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

175. Development of a High-Throughput Screening Platform for BSL-4 Pathogens.

Bersabeh Tigabu[1], Lynn Rasmussen[2], Nichole Tower[2], Saeed Mohammad[2], Barry Rockx[1], Alexander Bukreyev[1], E. Lucile White[2], James W. Noah[2]
[1]*The University of Texas at Galveston, Galveston, TX, United States,* [2]*Southern Research Institute, Birmingham, AL, United States*

176. Drug Design Studies on DENV RdRp.

Iuni M. L. Trist[1], Suzanne Kaptein[2], Pieter Leyssen[2], Johan Neyts[2], Andrea Brancale[1]
[1]*Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, United Kingdom,* [2]*Rega Institute for Medical Research, K. U. Leuven, Leuven, Belgium*

177. Novel Prodrugs of Acyclovir Cleavable by the Dipeptidyl Peptidase IV (DPP IV/ CD26) Enzyme.

Sonsoles Velázquez[1], Alberto Diez Torrubia[1], Silvia Cabrera[1], Sonia De Castro[1], Jan Balzarini[2], Mª José Camarasa[1]
[1]*Instituto de Quimica Medica (CSIC), Madrid, Spain,* [2]*Rega Institute for Medical Research, Leuven, Belgium*

178. Human Cytomegalovirus Resistance to Cyclopropavir Maps to a Base Pair Deletion in UL97 Which Results in a Viral Protein Lacking an Active Kinase Domain.

Laura E. Vollmer[1], Ellie D. Hall[2], Katherine Z. Borysko[3], Julie M. Breitenbach[3], Jiri Zemlicka[4], Jeremy P. Kamil[2], John C. Drach[3], Brian G. Gentry[1]
[1]*Drake University College of Pharmacy and Health Sciences, Des Moines, IA, United States,* [2]*Louisiana State University Department of Microbiology and Immunology, Shreveport, LA, United States,* [3]*University of Michigan Department of Biologic and Materials Sciences, Ann Arbor, MI, United States,* [4]*Wayne State University School of Medicine, Detroit, MI, United States*

179. Efficacy of Sida Cordifolia L. Extracts Against Herpes Simplex Virus Type 1 Infection *In Vitro* and *In Vivo*.

Ashish Wadhwani[1], Viral Patel[1], Kurokawa Masahiko[2], Vijayan Pottekad[1]
[1]*Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ootacamund, Tamil Nadu, India,* [2]*Department of Clinical Pharmacology, Kyushu University of Health and Welfare, Nobeoka, Miyazaki, Japan*

180. Immunopathogenesis of Different Common Emerging Infections: Enterovirus 71, Dengue Hemorrhagic Fever, SARS and A (H1N1)2009 Influenza.

Kuender D. Yang[1], Lin Wang[2], Jien-Wei Liu[2], Ron-Fu Chen[1], Chun-chen Li[2]
[1]*Chang Bing Show Chwan Memorial Hospital, Lu-gang, Changhua, Taiwan,* [2]*Kaohsiung Chang Gung Memorial Hospital, Niao-sung, Kaohsiung, Taiwan*

CAREER HAPPY HOUR

Chair(s): Tomas Cihlar, Ph.D.

SENS RESTAURANT

6:30 pm – 8:00 pm

Tuesday, May 14, 2013

CONTINENTAL BREAKFAST

MARKET STREET FOYER

7:30 am – 8:30 am

ORAL SESSION 5: HERPESVIRUSES AND POXVIRUSES

Chair(s): Rhonda Cardin, Ph.D. and Sophie Duraffour, Ph.D.

GRAND BALLROOM BC

8:30 am – 10:00 am

8:30 am **181. The Reverse Transcriptase Inhibitor Tenofovir (TFV) Also Targets the Herpes Simplex Virus (HSV) DNA Polymerase.**

Graciela Andrei, Sarah Gillemot, Robert Snoeck

Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

8:45 am **182. Selection and Recombinant Phenotyping of a Novel CMX001 and Cidofovir Resistance Mutation in Human Cytomegalovirus.**

Mark Prichard[1], Nathan Price[1], Caroll Hartline[1], Randall Lanier[2], Scott James[1]

[1]University of Alabama at Birmingham, Birmingham, AL, United States, [2]Chimerix Inc., Durham, NC, United States

9:00 am **183. Efficacy of Tranylcypromine (TCP) Against Herpes Simplex Virus Type 2 in Murine and Guinea Pig Models.**

R. D. Cardin[1], D.C. Quenelle[2], F.J. Bravo[1], D.A. Pullum[1], J.L. Vogel[3], T.M. Kristie[3], D.I. Bernstein[1]

[1]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, [2]University of Alabama School of Medicine, Birmingham, AL, United States, [3]Molecular Genetics Section, Laboratory of Viral Diseases, NIH , Bethesda, MD, United States

9:15 am **184. Kay-2-41: a Novel Nucleoside Analogue Inhibitor of Orthopoxviruses.**

Sophie Duraffour[1], Kazuhiro Haraguchi[2], Jan Balzarini[1], Kaori Yamada[2], Hirochimi Tanaka[2], Joost J van den Oord[3], Graciela **Andrei**[1], Robert Snoeck[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, [2]Showa University, School of Pharmacy, Tokyo, Japan, [3]University Hospitals Leuven, Laboratory of Translational Cell and Tissue Research, Leuven, Belgium

9:30 am **185. Effects of Low and High Volume Intranasal Treatments with an Adenovirus Vectored Interferon (mDEF201) on Respiratory and Systemic Infections in Mice Caused by Cowpox and Vaccinia Viruses.**

Donald F. Smee[1], Min-Hui Wong[1], Brett L. Hurst[1], Jane Ennis[2], Jeffrey D. Turner[2]

[1]Utah State University, Logan, UT, United States, [2]Defyrus, Inc., Toronto, Ontario, Canada

9:45 am **186. Feline Herpesvirus Ocular Disease – Developing an Antiviral Ophthalmic Solution for Cats.**

Nesya Goris[1], Joeri Auwerx[1], Eleonóra Kiss[1], Jérôme Villers[1], Aino Billiet[1], Philippe Hansen[2], Johan Neyts[1,3]

[1]Okapi Sciences NV, Heverlee, Belgium, [2]ECVO Diplomate , Brussels, Belgium, [3]Rega Institute (KU Leuven), Leuven, Belgium

COFFEE BREAK

MARKET STREET FOYER

10:00 am – 10:30 am

ORAL SESSION 6: EMERGING INFECTIONS

Chair(s): Mike Bray, M.D. and Justin Julander, Ph.D.

GRAND BALLROOM BC

10:30 am – 12:30 pm

10:30 am **187. Expecting the Unexpected: Targeting Bat Coronaviruses in Preparation for Their Human Descendants.**

Rolf Hilgenfeld[1,2,3], Yibei Xiao[1,2], Shyla George[1,2], Daizong Lin[3], Hong Liu[3], Christian Drosten[4], Yuri Kusov[1,2], Qingjun Ma[1,2]

[1]Institute of Biochemistry, University of Lübeck, Lübeck, Germany, [2]German Center for Infection Research, University of Lübeck, Lübeck, Germany, [3]Shanghai Institute of Materia Medica, Shanghai, China, [4]University of Bonn Medical Center, Bonn, Germany

10:45 am **188. Novel Inhibitors of SARS-CoV Entry Acting by Three Distinct Mechanisms.**

Adeyemi O. Adedeji[1,3], William Severson[5], Colleen Jonsson[6], Susan R. Weiss[4], Stefan G. Sarafianos[1,2]

[1]Christopher S. Bond Life Sciences Center, Department of Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia, MO, United States, [2]Department of Biochemistry, University of Missouri, Columbia, MO, United States, [3]School of Veterinary Medicine, University of California, Davis, CA, United States, [4]University of Pennsylvania School of Medicine, Philadelphia, PA, United States, [5]Southern Research Institute, Birmingham, AL, United States, [6]Center for Predictive Medicine for Bio-Defense and Emerging Infectious Diseases, University of Louisville, Louisville, KY, United States

11:00 am **189. A Novel Class of Host Mediated Antiviral Drugs Demonstrate Potent Inhibition of Dengue Virus Type 2.**

Ikenna M. Madu[1], Shari Kaiser[1], Myra Wang[1], Kerry Fowler[1], Sowmya Pattabhi[2], Micheal Gale Jr.[2], Shawn P. Iodonato[1], Kristin M. Bedard[1]

[1]Kineta Inc., Seattle, WA, United States, [2]University of Washington, Seattle, WA, United States

11:15 am **190. A Novel, Convenient and Robust Reverse Genetic System for the Production of a DNA-Based Live Attenuated Vaccine Against the Yellow Fever Virus.**

K. Dallmeier, J. Neyts

Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

11:30 am **191. Structure of Norovirus RNA-Dependent RNA-Polymerase in Complex with Three Naphthalene Derivates Inhibitors.**

Eloise Mastrangelo[1,2], Delia Tarantino[2], Romina Croci[2], Margherita Pezzullo[1,2], Martino Bolognesi[1,2], Mario Milani[1,2]

[1]CNR-Biophysics Institute, Milano, MI, Italy, [2]University of Milano, Department of Biosciences, Milano, MI, Italy

11:45 am **192. The Nucleoside Analogue 2'-C-Methylcytidine Inhibits Norovirus Replication *In Vitro* and Protects Against Virus Induced Diarrhea and Mortality in a Mouse Model.**

Joana Rocha-Pereira[1], MSJ Nascimento[1], Johan Neyts[2], Dirk Jochmans[2]
[1]*L. Microbiologia, D. Ciências Biológicas, Faculdade de Farmácia and Centro de Química Medicinal, Universidade do Porto, Porto, Portugal,* [2]*Rega Institute for Medical Research, University of Leuven, Leuven, Belgium*

12:00 pm **193. Identification of Acute Respiratory Failure and Long-Term Motor Function Deficits as Therapeutic Targets for Arboviral Encephalitides.**

John D. Morrey, Hong Wang, Venkatraman Siddharthan, Kyle K. Kessler, Jeffery O. Hall, Justin G. Julander
Utah State University, Logan, UT, United States

12:15 pm **194. Prevention of Lethal Rift Valley Fever Virus Disease by Post-Exposure Administration of MP-12 Vaccine Derivatives Lacking NSs.**

Brian B. Gowen[1,2,3], Kevin W. Bailey[1,2], Dionna Scharton[1,2], Zachary Vest[1,2], Jonna B. Westover[1,2], Ramona Skirpstunas[4], Tetsuro Ikegami[5,6,7]
[1]*Department of Animal, Dairy, and Veterinary Sciences,* [2]*Institute for Antiviral Research, and* [3]*School of Veterinary Medicine, Utah State University, Logan, UT, United States,* [4]*Department of Agriculture and Food, State of Utah, Logan, UT, United States,* [5]*Department of Pathology, The University of Texas Medical Branch, Galveston, TX, United States,* [6]*Sealy Center for Vaccine Development, The University of Texas Medical Branch, Galveston, TX, United States,* [7]*Center for Biodefense and Emerging Infectious Diseases, The University of Texas Medical Branch, Galveston, TX, United States*

Wednesday, May 15, 2013

CONTINENTAL BREAKFAST

MARKET STREET FOYER

7:30 am – 8:30 am

ORAL SESSION 7: MINI-CHEMISTRY SYMPOSIUM – PRODRUGS AS A TOOL IN DRUG DISCOVERY AND DEVELOPMENT

Chair(s): Michael Sofia, Ph.D. and Chris Meier, Ph.D.

GRAND BALLROOM BC

8:30 am – 11:30 am

8:30 am **195. A Case for Prodrugs.**

Valentino Stella, Ph.D.
University of Kansas

9:00 am **196. Rational Design of Nucleoside Phosphonates for Intracellular Delivery Using Lipid Conjugation.**

Randall Lanier, Ph.D.
Chimerix Inc.

9:30 am **197. Successful Application of Phosphate Prodrug Methodology to an HIV Attachment Inhibitor for the Treatment of HIV-1 Infection.**
John Kadow, Ph.D.
Bristol-Myers Squibb

COFFEE BREAK

MARKET STREET FOYER
10:00 am – 10:30 am

10:30 am **198. Design and SAR of Amidate Prodrugs for Acyclic and Cyclic Nucleoside Phosphonate Antivirals: the Discovery of GS-7340 and GS-9131.**
Richard Mackman, Ph.D.
Gilead Sciences

11:00 am **199. Prodrugs of Nucleosides and Nucleotides for the Treatment of HCV Infection: An Overview.**
Mike Sofia, Ph.D.
Oncore Biopharma, Inc

BUSINESS MEETING

Chair(s): Phil Furman, Ph.D.
GRAND BALLROOM BC
12:30 pm – 1:00 pm

ORAL SESSION 8: CLINICAL SYMPOSIUM

Chair(s): Joseph Colacino, Ph.D. and Randall Lanier, Ph.D.
GRAND BALLROOM BC
1:00 pm – 3:30 pm

1:00 pm **200. Clinical Development of Sovaprevir and ACH-3102: Two 2nd Generation Direct-Acting Anti-HCV Agents.**
Hetal Kocinsky, M.D.
Achillion Pharmaceuticals

1:30 pm **201. Preclinical and Clinical Studies of Miravirsen, a Novel Anti-HCV Therapeutic Targeting the Host Factor miR-122.**
Amy Patick, Ph.D.
Santaris Pharma

2:00 pm **202. ALS-2200/VX-135, and the Role of Nucleoside Analogs in the Treatment of Chronic Hepatitis C.**
John Fry
Alios BioPharma

2:30 pm **203. Cenicriviroc, a Novel, Once-Daily, Potent Dual CCR5 and CCR2 Antagonist Under Investigation for Treatment of HIV Infection.**
Eric Lefebvre, M.D.
Tobira Therapeutics

3:00 pm **204. Star Study: Single Tablet Regimen Rilpivirine/Emtricitabine/Tenofovir DF Is Non-Inferior to Efavirenz/Emtricitabine/Tenofovir DF in ART-Naïve Adults.**

Calvin Cohen[1], David Wohl[2], Jose Arribas[3], Keith Henry[4], Hui Wang[5], Danielle Porter[5], Shampa De-Oertel[5], Damian McColl[5]

[1]Community Research Initiative of New England, Boston, MA, United States, [2]Univ. of North Carolina , Chapel Hill, NC, United States, [3]Hospital Universitario La Paz, Madrid, Spain, [4]Hennepin County Medical Center, Minneapolis, MN, United States, [5]Gilead Sciences, Foster City, CA, United States

3:15 pm **205. CMV Resistance Profile of CMX001.**

Randall Lanier[1], Scott Foster[1], Sunwen Chou[2], Mark Prichard[3], Scott James[3], Tom Brundage[1], Herve Mommeja-Marin[1], Michelle Berrey[1]

[1]Chimerix Inc, Durham, NC, United States, [2]Oregon Health and Science University, Portland, OR, United States, [3]University of Alabama, Birmingham, AL, United States

SOFOSBUVIR-BASED REGIMENS FOR THE CURE OF CHRONIC HEPATITIS C

Phillip Pang, M.D. *Gilead Sciences, Foster City, CA, United States*

GRAND BALLROOM BC

3:30 pm – 4:00 pm

COFFEE BREAK

MARKET STREET FOYER

4:00 pm – 4:30 pm

ORAL SESSION 9: LATE BREAKER AND SHOTGUN POSTER PRESENTATIONS

Awardees to be Announced

Chair(s): Katherine Seley-Radkey, Ph.D. and Jennifer Moffat, Ph.D.

GRAND BALLROOM BC

4:30 pm – 5:30 pm

ICAR BANQUET RECEPTION

MARKET STREET FOYER

7:00 pm – 7:30 pm

ICAR BANQUET AND PROGRAM

BALLROOM B/C

7:30 pm – 9:30 pm

Join us for ICAR's Inaugural Women in Science Roundtable

Tuesday, May 14 from 12:30 – 2:30 pm

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This session will address the challenges and opportunities encountered by female scientists while navigating the twists and turns of career progression in todays' environment. Come talk to scientists in the industry, government and academic fields.

This roundtable will utilize the "speed dating" approach with small group conversations to address the following:

- **Do Super-Women Exist:** How to balance work and family through all life stages
- **Where do I Go From Here:** Maximize the benefits from the mentor/mentee relationship
- **Negotiation:** Tips on how to secure a mutually advantageous outcome without selling yourself short.
- **Is There a Glass Ceiling Left to Crack:** How to manage work force equality
- **Awards and Recognition:** Are they worth it and how to get on the short list?
- **Goals, Goals, Goals:** Learn to self-promote effecitvely to achieve your professional goals

MODERATORS:

- **Graciela Andrei,** *Rega Institute*
- **Heather Greenstone,** *NIAID*
- **Rhonda Cardin,** *Cincinnati Children's Hospital Medical Center*
- **Amy Patick,** *Pharmaceutical and Scientific Consultant*
- **Anneke Raney,** *Gilead Sciences*
- **Karen Watson-Buckheit,** *ImQuest BioSciences*
- **Jennifer Moffat,** *SUNY Upstate Medical University*

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If you have any questions about the roundtable or registering for the conference, please contact the ICAR registration desk.

Abstracts

POSTER SESSION 1: RETROVIRUSES, HEPATITIS VIRUSES, RESPIRATORY VIRUSES, AND ANTIVIRAL METHODS

4:30 pm – 6:30 pm

GRAND BALLROOM A and GRAND FOYER

23 The Effect of Lopinavir / Ritonavir an Antiretroviral Drug on the Antimalarial Activity of Artemether or Artemether / Lumefantrine in a Mouse Model of *Plasmodium berghei*

Oyindamola / O. Abiodun[1], John /A. Akinbo[2], Olushola /D. Ojurongbe[2]

[1]*Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan., Ibadan, Nigeria,* [2]*Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Oshogbo , Oshogbo, Nigeria,* [3]*Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Science, Ladoke Akintola University of Technology, Oshogbo, Oshogbo, Nigeria*

Malaria and HIV are important health problems. Lopinavir/ritonavir (LR) is a protease inhibitor that is used for the treatment of HIV while artemether/lumenfantrine (ART/LUM) is an artemisinin based combination therapy for the treatment of malaria. The possibility of drug interactions occurring during the treatment of malaria infection in HIV patients receiving antiretroviral drugs is very high and data on drug-drug interaction is limited. Thus this study evaluated the effect of LR on the antimalarial activity of artemether (ART) or ART/LUM in a mouse model of *Plasmodium berghei*. In total, ninety-five (95) mice weighing between 20-22g were inoculated intravenously with 1.0 x10^7 parasitized red blood cells and used for the study. A dose finding study of the efficacy of LR was done in order to select the appropriate dose for the interaction study. Treatment with 80/20 and 160/40 mg/kg of LR resulted in a two-fold decrease in parasitemia compared with untreated control ($p<0.05$) between days 3-10 post-inoculation. Despite initial suppression of parasite growth recrudescence of infection occurred and parasitemia increased in all animals treated with the selected doses of LR alone. Following the dose finding study of the efficacy of LR, 80 /20 mg/kg lopinavir/ritonavir was selected for the interaction study. The 50% effective dose (ED_{50}) of artemether alone on days 4 and 5 post infection was 0.80 ± 0.15 mg/kg and 2.18 ± 0.75 mg/kg respectively. Combination of a fixed dose of LR (80 mg/kg / 20 mg/kg) with graded doses of artemether in the treatment of mice infected with *P. berghei* did not significantly modified the ED_{50} values of artemether (0.88 ± 0.40 mg/kg and 3.53 ± 1. 09 mg/kg on days 4 and 5 post infection respectively). Treatment with a standard dose of ART/LUM or combination of ART/LUM with 80mg/kg kaletra resulted in complete suppression of parasite growth. Co-administration of LR with ART/LUM resulted in lower survival of experimental animals in comparison to those treated with standard dose of ART/LUM alone. The clinical implications for the toxicity observed in this study needs to be further investigated. **Keywords**: *Plasmodium berghei*, lopinavir / ritonavir (kaletra®), artemether, artemether/lumefantrine

24 Compounds Synthesized with a Novel Scaffold of (1,5)-di-Substituted Non-nucleosidic Uracil Inhibit Foci Formation by Hepatitis C Virus (HCV)

Abdullah A. Awadh[1], Helen S. Gureeva[2], Mikhail S. Novikov[2], Luis M. Schang[1]

[1]*Departments of Biochemistry and of Medical Microbiology and Immunology and Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Canada,* [2]*Volgograd Medical Scientific Center, Volgograd, Russia*

Several 5-substituted pyrimidine nucleosides have potent antiviral activities. In comparison, much less is known about 5-arylsubstituted non-nucleosidic pyrimidines. One of us (Novikov et al, 2010) has previously described the antiviral activity of 1-benzyl 5-arylamino uracil derivatives against HIV-1 and EBV in cell culture. We have

now synthesized 21 new derivatives and screened them for antiviral activity in cell culture. We screened a panel of DNA and RNA viruses, enveloped or not, with nuclear or cytoplasmic replication. None of the compounds had major inhibitory effects on HSV-1, VSV, influenza A virus, poliovirus or vaccinia virus. HCV replication was assessed using HCV_{cc} genotype 2a (JFH1) and the Huh7.5 hepatoma cell line. We evaluated foci formation at 72h after infection by immunocytochemistry with anti-HCV core antibody. Test compounds were added to the infected cells after removing the inoculum following a 4h adsorption at 37˚C. Four derivatives inhibited foci formation at low micromolar concentrations. Z197 and Z214 inhibited HCV_{cc} (JFH1) foci formation at the lowest concentrations (IC_{50}, 5.3 and 5.7 μM, respectively). The two other derivatives, Z246 and Z264, inhibited it only at higher concentrations (IC_{50}, approximately 20 μM). Structure-activity-relationship-guided optimization led to a derivative that inhibited HCV_{cc} (JFH1) foci formation at approximately 2-fold lower concentrations, Z304 (IC_{50}~ 2.0 μM). All compounds precipitated at high concentrations, which limited the selectivity index to the 10-50 range. None of the compounds inhibited HCV infectivity when the virions were pre-exposed to the compounds before infection. Likewise, pre-treatment of the Huh7.5 cells for one, two, or three days prior to infection had no effect on foci formation. In summary, we have identified a novel non-nucleosidic 1-benzyl 5-arylamino uracil scaffold that includes compounds which inhibit HCV_{cc} (JFH1) foci formation at the low micromolar range.

25 Computer-aided Discovery and Synthesis of Novel Anti-HCV Compounds

Marcella Bassetto[1], Pieter Leyssen[2], Johan Neyts[2], Andrea Brancale[1]
[1]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff, United Kingdom, [2]Rega Institute for Medical Research, Leuven, Belgium

Hepatitis C virus (HCV) is a major cause of chronic liver disease, leading to the development of hepatic steatosis, fibrosis, cirrhosis and hepatocellular carcinoma. The virus modifies the intracellular environment to promote its persistence within the liver, and its multi-factorial immune evasion capacity strongly impairs many treatment attempts. A vaccine is currently not available, while the standard of care used to be effective in only 50% of treated patients. The first specific anti-HCV drugs have been recently approved, while new classes of targeted agents are under clinical trials/investigation. Nevertheless, improved treatment strategies are needed, in order to bypass the rapid emergence of resistance. All the viral non-structural proteins are a possible target for the identification of novel and selective antivirals. Among them, the NS3 helicase is still underexploited, with no known inhibitor under pre-clinical or clinical development. This enzyme plays a crucial role in the virus life cycle: it catalyses the separation of double-stranded RNA strands, which is essential for genome amplification and translation.

Different computer-aided techniques were employed to identify, and subsequently synthesise, potential novel small-molecule inhibitors of its enzymatic activity. A structure-based virtual screening of commercially available drug-like compounds was performed on the main nucleic acid binding site, and a first series of candidates was evaluated in the HCV replicon system, yielding a primary hit with low μM activity. Secondly, the model of the one known inhibitor co-crystallised with this enzyme was used as starting point for a shape-comparison screening of small molecule libraries. A second series of compounds was selected and evaluated for anti-HCV activity, and one molecule was shown to inhibit the viral replication at low μM concentration. Based on the scaffolds of these hit structures, two series of derivatives were designed and synthesised: different compounds with low μM activity were identified as potential anti-HCV agents.

26 Small Molecule Agonists of the RIG-I Pathway and Their Broad Spectrum Inhibition of Hepatitis Viruses; Including Hepatitis C and Hepatitis B Virus

Kristin Bedard[1], Myra Wang[1], Kerry Fowler[1], Wazir Abdullahi[1], Michael Gale, Jr.[2], Shawn Iadonato[1]
[1]KINETA, Inc., Seattle, USA, [2]University of Washington, Seattle, USA

We report the identification of novel, drug-like small molecule agonists of the RIG-I innate immune pathway that demonstrate effective antiviral activity against both Hepatitis C and Hepatitis B viruses. These drugs cause a significant inhibition of viral protein, nucleic acid and the production of infectious virus in cell based models of viral hepatitis. Hepatitis C virus is a highly successful virus infecting nearly 200 million people worldwide and

causing a chronic lifelong infection in approximately 75% of acutely infected subjects. Similarly, Hepatitis B virus is a major health concern worldwide and it is estimated that one third of the global population is infected with 5% sustaining a chronic lifelong infection of which a quarter of people will develop serious liver disease. Recent drug development efforts have focused on antiviral products that directly target key viral enzymes and despite the increasing occurrence of co-infections little attention is spent on developing broad spectrum hepatitis antivirals. Drugs that modulate and enhance innate immunity would display broad antiviral activity, immune-enhancing efficacy and an ability to overcome virus countermeasures, while remaining insensitive to the rapid evolution of drug resistance that plagues conventional small molecule therapies. A key pathway that is responsible for mediating the innate immune response to RNA virus infection involves activation of RIG-I and targeting this pathway has successfully lead to the identification of agonist molecules that are highly potent and broadly active antiviral molecules. The novel antiviral compounds described activate RIG-I responsive promoters by mediating nuclear translocation of IRF-3 and display highly potent antiviral activity against Hepatitis C virus and Hepatitis B virus. The antiviral drugs have been optimized through medicinal chemistry resulting in potent derivatives with a high therapeutic index. Additionally, these host mediated drugs have shown potent activity when used in combination with suboptimal doses of direct-acting HCV drugs.

27 Anti-HIV Metal Complexes of Pyridine-Fused Macrocyclic Polyamines Targeting the Cellular HIV Co-receptors CXCR4 and CCR5

Thomas W. Bell[1], Sunil Hamal[1], Dana Huskens[2], Thomas D'huys[2], Dominique Schols[2]
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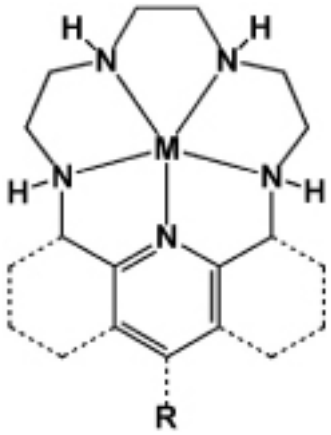
A number of macrocyclic polyamines and their metal complexes are demonstrated to have anti-HIV activity. A well-described class is the bicyclams (e.g. AMD3100) and their metal complexes that act as potent HIV entry inhibitors via specific binding to the cellular HIV co-receptor CXCR4. By synthesizing and screening various pyridine-fused macrocyclic polyamines, we have discovered lead compounds that act as HIV entry inhibitors by interacting with CXCR4 and some also with the second cellular HIV co-receptor, CCR5. The structures of these novel compounds are represented in the general figure shown below. They consist of pyridine, 4-methylpyridine, tetrahydroquinoline, or octahydroacridine rings fused to a 1,4,7,10,13-pentaazacyclopentadecane ring. The metal-free ligands and their metal complexes (M = Mn^{II}, Cu^{II}, Fe^{III}, or Zn^{II}) have various potencies in inhibiting X4 HIV-1 IIIB and HIV-1 NL4.3 replication in MT-4 $CD4^+$ T cells. Some also have significant antiviral activity against various X4 and R5 viruses when evaluated in PBMCs, TZM-bl cells and monocytes/macrophages. For example, compound SH38 consisting of the $FeCl_3$ complex of the octahydroacridine-fused macrocycle shows submicromolar IC_{50} values for inhibition of replication of X4 and R5 HIV viruses.



28 Synthesis and Evaluation of New Hepatitis C Virus NS3 Protease Inhibitors with an Azetidine Ring at the P2 Position

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Hepatitis C virus (HCV) is a major health problem affecting an estimated 170 to 200 million individuals worldwide and its infection is a leading cause of chronic liver disease in the United States. Despite the existing treatments involving pegylated interferon-α (IFN) and ribavirin (RBV), with or without protease inhibitors (PI) boceprevir

(Victrelis) and telaprevir (Incivek), their limited efficacy and side effects emphasize the need for additional and improved therapeutic agents. Since the discovery of both telaprevir and boceprevir, numerous structural modifications have been investigated at various positions of these peptidomimetics. Herein, we detail the introduction of new azetidine and spiroazetidine moieties at the P2 position of the peptidomimetic. Synthesized compounds were evaluated for inhibition of HCV RNA replication in Huh7 cells using a subgenomic HCV replicon system. Cytotoxicity in Huh7 cells was determined simultaneously with anti-HCV activity by extraction and amplification of both HCV RNA and ribosomal RNA (rRNA). The most active compounds, which displayed no toxicity in Huh 7 cells, were further profiled by assessing their cytotoxicity in CEM, Vero (African green monkey kidney cells), and human PBM (peripheral blood mononuclear) cells. Among the 17 analogs prepared, several displayed anti-HCV potency in the micromolar to submicromolar. Unfortunately, cytotoxicity in one of more cell based systems discouraged further development of this new series of HCV NS3 PI.

29 Hit-to-Lead Optimization in a Series of Tetrahydroquinolines for the Treatment of HCV

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BACKGROUND: Hepatitis C virus infection is a leading cause of cirrhosis, liver cancer, and liver transplantation worldwide. An important variable for HCV patients is the genotype (1-6) of HCV with which they are infected due to varying responses to current HCV treatments. In the past decade, intensive efforts have focused on the discovery of novel antiviral agents to improve both the tolerability and efficacy of HCV therapy, as compared with the current standard treatment of peginterferon, ribavrin and telaprevir or boceprevir. Although there have been numerous studies of small molecules designed to treat HCV with varying modes of action, the majority of these therapeutics are limited to activity against genotype 1 infection. Herein we report the antiviral structure activity relationship (SAR) of a series of novel small molecules that exhibit antiviral activity against infectious HCV 1b and 2a grown in cell culture (HCVcc). METHODS: A series of substituted tetrahydroquinolines were prepared. Antiviral activity was measured with infectious HCV in Huh-7 cells. RESULTS: An in-house screening campaign followed by biological characterization identified tetrahydroquinolines as small molecules that inhibit HCV infectivity. The hit compound had moderate potency (HCVcc EC_{50} 2a = 652 nM) with poor physicochemical properties (Log D 5.0) and poor stability in human microsomes (T½ <5 min). A series of novel tetrahydroquinolines with various C-3 and C-4 moieties was synthesized and the SAR with respect to infectivity inhibition in HCVcc, microsomal stability and lipophilicity was established. Initial exploration at C-3 ester led to improved microsomal stability and a 2-fold improvement in potency (HCVcc 2a EC_{50}= 300 nM, T½= 62 min). Additional optimization at C-4 further enhanced both potency and physio-chemical properties. CONCLUSIONS: The SAR exploration and optimization of a hit molecule identified from an in-house screening library led to the identification of a lead compound with potent pan-genotypic activity (HCVcc EC_{50} 2a = 23 nM, 1b/2a = 24 nM), improved physicochemical properties (Log D 3.1) and improved microsomal stability (T½= 48 min). CONTRIBUTORS: Eda Canales, Kerim Babaoglu, Rudolf Beran, Caroline Bush, Michael Clarke, Sara Eng, Robin Higgins, Tetsuya Kobayashi, Ruben Martinez, Philip Morganelli, Bernard Murray, Roland Saito, Hung Trinh, Matthew Paulson, and Scott Lazerwith.

30 Phenothiazines Inhibit HCV Entry by Increasing the Fluidity of Cholesterol-rich Membranes

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Despite recent progress in the development of direct acting antiviral agents against hepatitis C virus, more effective therapies are still urgently needed. We and others have previously identified three phenothiazine compounds as potent HCV entry inhibitors. In this study, we show that phenothiazines inhibit HCV entry at the step of virus-host cell fusion by intercalating into cholesterol-rich domains of the target membrane and increasing membrane fluidity. Perturbation of the alignment/packing of cholesterol in lipid membranes likely increases the energy barrier needed for virus-host fusion. A screening assay based on the ability of molecules to selectively increase the fluidity of

cholesterol-rich membranes was subsequently developed. One compound, topotecan that emerged from the library screen is able to very potently inhibit the fusion of liposomes with cell culture-derived HCV (HCVcc). These results yield new insights into HCV infection and provide a platform for the identification of new HCV inhibitors.

31 Anti-adhesive Properties of Plant Extracts Cystus052 und Ladania067 as a Broad Range Antiviral Mechanism Against Respiratory Viral Pathogens

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Severe and acute respiratory infections including those resulting in pneumonia are the main infectious diseases globally accounting for an estimated 3.9 million death per year. The emergence of new and highly dangerous respiratory pathogens, such as SARS coronavirus or highly pathogenic avian influenza viruses highlight the urgent need for safe and effective antiviral therapeutics with broad activity. In this context, plant products from traditional medicine came into focus of antiviral research. Cystus052 is a polyphenol rich plant extract from a special variety of Cistus incanus that exhibits a broad antiviral activity *in vitro* and *in vivo* against all tested subtypes of influenza viruses, but also against other respiratory pathogens such as rhinoviruses and adenoviruses. The antiviral action is based on an interaction of the extract ingredients with the viral surface, that prevents subsequent binding and penetration of the pathogen into the host cells. At the same time host cells seem to be inert to the action of the extracts. Cystus052 treatment neither effected cell morphology nor proliferation, metabolism or viability of cells. Furthermore, Cystus052 did neither trigger receptor-mediated signals in the cell, nor inhibited the activation of these processes by their physiological ligands. Thus, Cystus052 functions in a non-pharmacological anti-adhesive manner. Due to this non-specific attack no viral resistance to the extract components was observed. Recently, Ladania067, a preparation from the leaves of a wild growing Ribes nigrum folium variety, was identifed as another plant extract with broad and strong antiviral activity which follows similar mechanisms of action. Thus, anti-adhesive acting plant products are a promising option for the treatment of respiratory viruses and may also be considered for prophylaxis.

32 Novel HCV Binding, Fusion and Envelope Fluidity Assays Identify the Antiviral Mechanisms of the Natural Products Epigallocatechin Gallate and Curcumin

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Epigallocatechin gallate (EGCG) and curcumin are two of the many natural products with antiviral activities against hepatitis C (HCV) and other viruses. The antiviral mechanisms of many natural products, including EGCG and curcumin, remain mostly unclear. To characterize these mechanisms against HCV, we have developed assays to test specific entry steps, using infectious HCVcc (JFH1) and susceptible Huh7.5 cells. To evaluate binding, HCV virions fluorescently labeled with octadecyl rhodamine B chloride (R18) were incubated with Huh7.5 cells at 4C. Unbound virions were washed away before cells and bound virions were lysed. Virion binding was evaluated by the fluorescence intensity in the lysates. To evaluate fusion, R18-labeled virions were attached to target cells at 4C. Unbound virions were washed away. Fusion of bound virions was then triggered by decreasing the pH and increasing the temperature and was monitored by fluorescence dequenching. To test for potential effects on envelope fluidity, we examined the fluorescence polarization of diphenylhexatriene (DPH). EGCG and curcumin inhibited the infectivity of HCV (IC_{50}, 1.3 μM and 7.5 μM, respectively). We then identified the specific entry steps inhibited by EGCG and curcumin. EGCG inhibited HCV binding (IC_{50}, 70 μM), but not fusion, to Huh7.5 cells. EGCG had no effects on the fluidity of the HCV envelope. We proposed that EGCG inhibits the primary low-affinity attachment of HCV by competing with cellular glycosaminoglycans for virion binding. Curcumin, in

contrast, inhibited both the binding and the fusion of HCV to cells (IC_{50}, 15 μM and < 20 μM, respectively). These effects are consistent with a potential modulation of membrane fluidity, as appropriate membrane fluidity is crucial for high affinity binding and fusion. Consistently with this expectation, curcumin decreased the fluidity of the HCV envelope. In conclusion, we developed novel binding, fusion and envelope fluidity assays for HCV. We then used these assays to identify the specific entry steps inhibited by two small molecule natural product inhibitors of HCV entry, EGCG and curcumin, which inhibit attachment and modulate membrane fluidity, respectively.

33 Rapid and Convenient Assays to Assess Potential Inhibitory Activity on *In Vitro* Hepatitis A Replication

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The hepatitis A virus (HAV) is a faeco-orally transmitted picornavirus and is one of the main causes of acute hepatitis worldwide. The virus is endemic in most developing countries, but also causes occasional outbreaks in industrialized regions. Efficient and safe vaccines are available. Nevertheless, it would be important to have antiviral drugs targeting HAV at hand to treat severe cases of fulminant hepatitis, to contain outbreaks and to stop the potential spread of vaccine-escape variants. Also, such drugs could be used to shorten the period of illness and to decrease associated economical costs. As a first step towards such drugs, three different antiviral assays were developed for the *in vitro* screening of inhibitors of HAV of which (i) a cytopathic effect reduction assay suitable for medium-to-high throughput screening and (ii) two virus yield reduction assays (based on quantification of viral RNA) for genotypes IB and IIIA. The assays were validated for antiviral studies with interferon-alpha (IFNα) and amantadine HCl, two known inhibitors of HAV replication. IFNα effectively inhibited HAV replication, whereas the activity of amantadine HCl appeared to be strain-dependent. Employing these assays, we assessed the effect of the known enterovirus inhibitors pleconaril, rupintrivir and enviroxime on HAV replication. Pleconaril exhibited some very moderate activity, the effect of rupintrivir proved to be strain-dependent. Enviroxime did not inhibit HAV replication, suggesting that phosphatidylinositol-4-kinase IIIβ is not crucial in the HAV life cycle.

34 Interferon Alpha and Ribavirin are Potent Inhibitors of Hepatitis E Virus Replication *in vitro*

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Hepatitis E virus (HEV) is among the most common causes of acute hepatitis worldwide. Four HEV genotypes are currently being recognized with genotypes 1 and 2 exclusively infecting humans and genotypes 3 and 4 being zoonoses with their main reservoir in domestic pigs. Mortality rates are particularly high in pregnant women (up to 25%). Moreover, HEV can cause chronic infections in immune compromised patients (e.g. after organ transplantation). Other than lowering immune suppression, successful treatment of HEV infections has been reported with pegylated interferon alpha (IFNα) and/or ribavirin. So far, no *in vitro* proof of the anti-HEV activity of IFNα and ribavirin has been reported. We developed an antiviral assay based on the recently reported Gaussia luciferase-expressing p6 HEV strain and confirmed in this assay the antiviral activities of IFNα (EC_{50} of 1.3+/-0.5 IU/mL) and ribavirin (EC_{50} of 3+/-2μM). One of the proposed mechanisms of action of ribavirin is depletion of GTP pools; therefore the anti-HEV activity of two other known GTP depletors, EICAR and mycophenolic acid (MPA), was assessed in the luciferase assay. Comparable activities were found for both compounds (EC_{50}'s of 0.115+/-0.007μM and 0.20+/-0.04μM respectively). Virus replication could be salvaged by the addition of exogenous guanosine to the culture medium. Next we determined the EC_{50}'s for GTP depletion by ribavirin, EICAR and MPA in the cells used. Preliminary results suggest a good correlation between GTP depletion and inhibition of HEV replication. Finally, we evaluated the effect of ribavirin and IFNα in a RT-qPCR-based virus yield assay and observed initial evidence of robust inhibition of virus replication in this model as well.

35 Novel Norbornane-based Nucleoside and Nucleotide Analogues and Their Antiviral Activities

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Nucleoside and nucleotide therapeutics represent important classes of drugs used in the treatment of viral diseases. Various locked nucleosides were reported in the past and some of them brought a significant promise for the treatment of infections caused by diverse viral pathogens. Recently, we introduced norbornane as one of the possible bicyclic systems that could serve as a conformationally locked substitute of the natural furanose ring and we showed that several 9-norbornylpurines exert significant activity against Coxsackieviruses (Picornaviridae). In our current work, we have turned our attention to the conformationally locked norbornane-based derivatives of abacavir, a commercially successful anti-HIV agent, and prepared a number of derivatives with the pseudosugar locked in North, South and also East conformation in order to investigate their potential to serve as antiviral agents. The target compounds were evaluated in our broad antiviral assay panel, which includes a spectrum of human and animal viral pathogens from various RNA-, DNA- and retrovirus families. Within these series, we have identified several interesting compounds with activities against HIV-1 and HIV-2, Feline herpes virus and influenza virus (H1N1). Furthermore, numerous 6-chloropurine and 2-amino-6-chloropurine derivatives enriched our library of anti-Coxsackievirus compounds. The synthesis and biological activities of these nucleoside and nucleotide derivatives will be discussed in detail. **Acknowledgement:** Authors are grateful to project CZ.1.07/2.2.00/28.0184 coming from European Social Fund. The synthetic studies were supported by Gilead Sciences, Inc. (Foster City, CA, USA) and subvention for development of research organization (RVO: 61388963). The virological studies were supported by grants of the KU Leuven.

36 Alpharetroviral Vectors Are Suitable Tools For Tre-recombinase Based HIV-1 Gene Therapy

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After 25 years of research and the introduction of antiretroviral therapy (ART), infection with human immunodeficiency virus type 1 (HIV-1) can still lead to the acquired immunodeficiency syndrome (AIDS), which ultimately results in the death of the infected individual. Recent antiviral approaches also include gene therapy of $CD34^+$ hematopoietic stems cells (HSC). A promising new tool for HIV-therapy could be Tre-recombinase, an enzyme that specifically recognizes and excises proviral DNA from the genome of HIV-infected cells (Sarkar et al. 2007).

A prerequisite for the use of Tre-recombinase in gene therapy is the efficient and safe gene delivery of the Tre coding sequence into target cells for subsequent faithful and stable Tre expression. Due to their neutral integration pattern recently developed replication incompetent self-inactivating (SIN) alpharetroviral vectors may be advantageous over currently used lentiviral vectors, particularly with respect to genotoxicity and splicing profiles (Suerth et al. 2012 Kaufmann et al. 2012).

In the present study, we constructed an advanced alpharetroviral vector for the transfer of the Tre-recombinase cDNA into HIV-1-infected cells. We demonstrate that Tre-encoding alpharetroviral vectors are able to transduce human cells without any toxic side effects and facilitate conditional and efficient transgene expression in HIV-infected (i.e. Tat expressing) cells. Furthermore, alpharetroviral-delivered Tre-recombinase shows antiviral activity *in vitro* and is able to reduce the proviral load in cell cultures, which are stably infected with HIV-1. In addition, we show that alpharetroviral vectors are able to transduce human primary $CD34^+$ HSC with similar efficiencies as lentiviral vectors and that these cells engraft in immune-compromised $RAG2^{-/-}\gamma c^{-/-}$ mice. Thus, our results reveal that alpharetroviral vectors are attractive and valuable tools for HIV-gene therapy.

37 HIV-Specific Promoters to Eliminate HIV-Infected Cells by Gene Therapy

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OBJECTIVES: Current antiretroviral therapies against HIV infection are unable eradicate the chromosomally integrated proviral genome. We are developing an HIV-specific promoter to drive the expression of suicide genes that would induce cell death in HIV-infected cells, but not in uninfected cells. Here we examined the expression of luciferase driven by the HIV promoter, LTR, and 5 mutant LTRs. Our aim is to design a promoter that is responsive to the HIV transcriptional activator, Tat, but not to cellular transcription factors in uninfected cells. METHODS: The full-length LTR and five progressively truncated versions of the promoter were generated using PCR-based cloning techniques, and designated LTR1–LTR6 (Bionexus). These promoters were inserted into the pGL3 Basic Vector encoding luciferase (Promega). These plasmids were transfected into HeLa cells, and HeLa-*tat*-III cells that constitutively express Tat, using the transfection reagent Metafectene (Biontex). Luciferase activity (relative light units (RLU)/ml cell lysate) was measured 48 h later, using the Luciferase Assay System (Promega). RESULTS: Luciferase expression from LTR1 containing the wild type HIV promoter, was 595 RLU/ml in HeLa cells, and 30,373 in HeLa-tat-III cells, showing the specific activation by Tat. In LTR2, the LTR modulatory region was truncated, but the NF-κB binding region was maintained. Luciferase expression from the LTR2 construct increased from 1,060 RLU/ml in HeLa cells to 108,187 RLU/ml in HeLa-tat-III cells, a 102-fold increase. In LTR3 without the NF-κB binding region the corresponding values were 498 and 25,387 RLU/ml. The other constructs resulted in much lower gene expression in both cell types. CONCLUSIONS: HIV-specific cell killing may be possible by generating a suicide gene construct driven by the LTR2 promoter. It is expected that the incorporation of LTR2, or an improved construct with higher Tat-specificity, into a lentiviral vector may lead to the therapeutic transduction of all HIV-harboring cells. *Supported by Research Awards 03-Activity-071 and 03-Activity-076 from the Arthur A. Dugoni School of Dentistry.*

38 Reduce the Risk in Vulnerability in HIV

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BACKGROUND; Before the people can reduce the risk and vulnerability to HIV, individuals and communities must understand the urgency to the epidemic. They must be given basic facts about HIV/aids, taught set of protective skills and offered access to appropriate services and products. METHODS A cross sectional community based survey have been conducted between January 2011 to April 2011 by interview of 419 heads of the households regarding the knowledge of HIV transmission means in Accra, Ghana RESULTS; Out of the interviewed 419 household heads,287(68.5%) were females.36%,28% and34.3% were in the age group of 40+,31-40 and15-30 respectively. The findings revealed that only21(5%)of study participants mentioned four ways of HIV transmission(unprotected sex ,mother to child, sharp materials and blood transfusion).On the other hand,63(15%) mentioned any three of the above route of transmission, whereas the majority ,209(50%) and 86(20.5%) mentioned two and one means of HIV respectively. On contrary about 40(10%) of interviewed heads of house hold mentioned hardly any of the transmission means. Males were about 2.4 times more likely to mention unprotected sex as one means of transmission than females CONCLUSION; In general, knowledge of residents of Medina in the capital town of Ghana about HIV transmission and prevention means was low. Appropriate HIV/Aids education means ought to be tailored to residents

39 Can Small Molecule Inhibition of the HIV gp41 603–609 Dicysteine Loop Inhibit Viral Replication and Reverse the HIV-Induced Elevation of cAMP and IL-10?

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The characterization of HIV-1 envelope glycoprotein gp41's contribution to HIV-1 pathogenesis with respect to its essential role in HIV-1 to host cell membrane fusion activity is well documented. However, gp41's contribution

to HIV infectivity with respect to its role as a unique immunosuppressive entity is not as well understood. Much of the evidence points to the involvement of a specific region of gp41 known as the immunosuppressive domain. Gp41's immunosuppressive domain, as defined by Yinghua Chen, lies between amino acids 583-599 and was determined to induce similar inhibitory effects on lymphocyte proliferation and MHC upregulation as human type 1 interferons (Chen, 1998). This similar activity was attributed to a homology existing between gp41's immunosuppresive domain and human IFN-α and IFN-β (Chen, 1998). In addition to the immunosuppressive region, the immunodominant domain, which lies just downstream of the immunosuppressive sequence, between amino acids 598-609, has also been implicated in contributing to HIV-1 infectivity by modulating the expression of cytokines and cAMP in monocytes (Speth, 2000). To more clearly define this activity and the region of gp41 involved, we performed a Z score analysis between the immunodominant sequence (aa 587-609) and the G-protein $G_i\alpha$ subunit and $G_i\alpha$ GTPase. We found homologies existing between gp41 (aa589-595, AVERYLK) and $G_i\alpha$ (aa12-17, AVER-SK), as well as between gp41 (aa597-611, QQLLGIWGCSGKLIC) and $G_i\alpha$ GTPase (aa36-50, IILLGAGESGKSTIV). For the latter comparison, a Z score of 3.0501 was found. We believe this evidence, coupled with previous evidence of gp41›s ability to induce upregulation of cytokines, is sufficient to establish gp41›s immunodominant domain, and specifically the dicysteine loop (aa 603-609 CGSKLIC) as a unique target for small molecule inhibition. Since recombinant HIV gp41 aa565-647 (which includes the 603-609 dicysteine loop) elevates both cAMP and IL-10, assays to determine whether small molecule inhibition of the loop will reverse the elevation of cAMP and IL-10 should be used – in addition to standard assays of viral replication (Barcova, 1999).

40 From the Discovery of New Inhibitors of Rhinovirus Replication Toward the Development of an Antiviral Against A Wide Range of Enteroviruses

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Human rhinoviruses (HRV) remain a significant public health problem as they are the major cause of both upper and lower respiratory tract infections. To date no vaccine or antiviral are available against these pathogens. Using a high-throughput yeast two-hybrid screening, we identified a six amino acid "hit" peptide, LVLQTM which acted as a pseudo-substrate of the viral 2A cysteine protease (2Apro) and inhibited its activity. This peptide was chemically modified with a reactive electrophilic fluoromethyl ketone group to form a covalent linkage with the nucleophilic active site thiol of the enzyme. Ex vivo and *in vivo* experiments showed that thus converted, LVLQTM was a strong inhibitor of HRV replication in both A549 cells and mice. To our knowledge, this was the first report validating a compound against HRV infection in a mouse model. Based on HRV-2 2Apro crystallographic data, a virtual docking model was then set up to predict the inhibitor binding mode into the ligand binding pocket of the enzyme. Sequence comparison between different 2Apro from HRV-A, -B and –C species revealed that aminoacid residues involved in the interaction with the inhibitor in our model are relatively well conserved.

If our peptide inhibitor seemed to be of general use against all HRV serotypes, its use for therapeutic purposes could be extended to other enterovirus-associated diseases since it was also active against Poliovirus 1 (PV-1) and Human Enterovirus 71 (HEV-71) 2A proteases. Moreover, comparison of the sequence of these proteases with the other proteases tested in this study for their interaction with LVLQTM revealed only minor differences. Therefore, this study opens new doors in the development of an antiviral against a wide range of enteroviruses.

41 An Innovative Approach for Multiplexed RSV Replicon Assay

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Respiratory syncytial virus (RSV) is an important human respiratory pathogen, and there is an urgent and unmet medical need for novel anti-RSV therapy. In antiviral drug discovery, cell-based sub-genomic replicon systems have been successfully used in compound library screening, antiviral mechanism of action studies, and to optimize antiviral potency. A replicon system containing a *Renilla* luciferase reporter gene was built for RSV to enable

identification of inhibitors targeting RSV replication. In this assay, compounds inhibiting viral replication resulted in a diminished luciferase signal. To eliminate compounds that produced a diminished luciferase signal as a result of affecting the viability of the mammalian cell line, rather than inhibiting RSV replication, a cytoxicity assay was run as a counter screen in parallel with the same mammalian cell line used for the RSV replicon assay. Given the fact that the *Renilla* luciferase reporter in the RSV replicon allows for live-cell readout, measurement of this could be made prior to measuring cytotoxicity with the same construct, and therefore we explored if these two assays could be combined in one single multiplex assay. Validation of this multiplex replicon assay was performed using a small sub-set of library collection compounds. Similar consistent and reliable results were generated, with comparable EC_{50} values obtained from both assays. In conclusion, we successfully developed a multiplex replicon assay which delivers both replicon and cytotoxicity readouts in a single assay format, leading to significant reduction in compound usage, assay cost and turnaround time. This assay format could also be applied to other antiviral assays, such as the infectious hepatitis C virus (HCV) assay which also uses the *Renilla* luciferase readout, and that is currently run separately from the cytotoxicity assay to support HCV drug discovery.

42 CYP24A1 Inhibitors and Vitamin D: A New Potential Anti-HCV Strategy?

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Important progress in hepatitis C virus (HCV) pharmaceutical development had been made in the last 20 years. Unfortunately, efficient non-toxic therapies remain an aspiration for the future. Therefore the slow down of the disease progression and the improvement of sustained virological response (SVR) rates are immediate targets for the short-term research.

Recently, an increasing number of clinical evidence has proved the risks of vitamin D (calitriol) deficiency in subjects suffering from chronic HCV infection, and supplementary vitamin D has been proposed as an addition to the usual treatment against the virus[1]. Patients with severe vitamin D insufficiency almost never reach SVR while those with regular vitamin D value or after receiving supplementation of calcitriol achieve an SVR rate in more then half the cases[2]. Numerous experimental evidence have shown anti-HCV activity of calcitriol results from three main actions: anti-inflammatory[3], anti-fibrotic[3] and anti-viral effect[4].

The growing body of clinical/experimental evidence makes calcitriol an optimal candidate against chronic HCV infection. Unfortunately, a therapy using calcitriol remains a challenge due to increased drug resistance as a consequence of the up-regulation of CYP24A1, which metabolises and inactivates calcitriol[5]. Moreover, the hyperlcaemia associated with an elevated dose of calcitriol, does not allow the use at a high vitamin D concentration. The use of CYP24A1 selective inhibitors could be the appropriate strategy to increase the lifetime and thereby the anti-HCV functions of calcitriol[6].

The aim of this project is to develop potent and selective inhibitors of CYP24A1 that could be used in the treatment of chronic HCV infection together with the canonical treatments and vitamin D supplementation in order to enhance the vitamin D levels and favour its anti-viral effects.

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43 Antiviral Activity of Sxtracts from Cistus Incanus and Ribes Nigrum Against Different Influenza Virus Strains

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Influenza, a respiratory disease caused by influenza viruses, still represents a major threat to humans and several animal species. Beside vaccination, only two classes of drugs are available for antiviral treatment against this

pathogen. For that reason there is a strong need for new effective antivirals against influenza viruses. Here, we tested two plant extracts from the leaves of the wild black currant (*Ribes nigrum folium*) and from *Cistus incanus* for their potential antiviral activity against different influenza virus strains *in vitro* and *in vivo*, including strains that are resistant to oseltamivir treatment. In the range of 0 - 1mg/ml both extracts showed no cytotoxic effect on A549 and MDCK cells. Both extracts were highly effective (EC_{50} value: 0,05 – 1 µg/ml) against H1N1pdm09, H1N1, H3N2 and influenza B virus. The extracts exhibited an antiviral effect when the virus was pre-incubated with the extracts prior to infection or when added directly after infection. No antiviral effect was found when infected cells were treated 2, 4 or 8 h after infection. This indicates that both extracts blocks a very early step in the virus infection cycle, most likely virus entry. In the mouse infection model we could demonstrate that an intranasal application of 500 µg of either extract inhibits progeny virus titers in the lung. We conclude that both extracts may be a promising source for the identification of new molecules with antiviral functions against influenza virus.

44 *In Vitro* Evaluation of the HIV Therapeutic IQP-0410 Through Transdermal Patch Delivery

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The pyrimidinedione IQP-0410 is a highly potent non-toxic, dual-acting HIV nonnucleoside reverse transcriptase inhibitor (NNRTI) that targets both virus entry and reverse transcription, and is proposed to provide therapeutic opportunities not available with many other NNRTIs due to its high therapeutic index and novel dual mechanism of action. Through conventional oral administration IQP-0410 and the other PYD analogs are subjected to extensive first pass metabolism. Therefore, transdermal patches are being investigated and developed for long term IQP-0410 delivery which would avoid this first pass metabolism, and significantly improve patient compliance through a weekly dosing regimen. IQP-0410 was formulated into an ethyl cellulose / HPMC based patch via a solvent cast process. *In vitro* characterizations evaluating transdermal patch physicochemical properties, drug release and delivery, and formulation stability were conducted alongside *in vitro* antiviral, mechanism of action, and toxicity assays to evaluate the efficacy and toxicity of formulated IQP-0410.

Flexible, strong transdermal patches loaded with 2% (w/w) of the HIV therapeutic IQP-0410 were produced that have no irritation to *ex vivo* skin tissue and no *in vitro* toxicity to HIV target cells CEM-SS and HeLa-CD4-LTR β-gal cells. When applied to the skin model, IQP-0410 was delivered at a rate of 0.661 ± 0.0027 mg/cm^2/hr. Over a 3 day application to the skin model, the *in vitro* EC_{50} of the permeated IQP-0410 was 2.557 ± 0.401 nM (CEM-SS) and 0.0199 ± 0.001 nM (PBMC). In sealed light-protected packages, the transdermal patches demonstration no significant degradation at regular (35°C / 65%RH) and accelerated stability conditions (40°C / 75%RH) for over 3 months. IQP-0410 is a promising, highly potent HIV therapeutic currently being prepared for IND submission. Transdermal patches represent a drug delivery system that would allow the non-toxic delivery of IQP-0410 at therapeutic levels over extended periods of time with a single application. Such a controlled delivery system may be necessary and advantageous over traditional therapeutic delivery to improve the successful use of HIV therapeutic products.

45 Development of Influenza Virus Inhibitors with a Higher Genetic Barrier to Resistance

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Influenza virus infects over 500 million people annually resulting in variable degrees of systemic symptoms, ranging from mild fatigue to respiratory failure and death. Even when the predictive strains of the trivalent annual vaccine are actually predominant in the population during the typical influenza virus season, the vaccine is only about 64% protective in immunized patients. Only four FDA-approved antivirals (AMT, RMT, OSC, ZNV) are available for treatment and they have a short window of opportunity to begin treatment following infection in order reduce the duration and severity of illness. Throughout the years, many of the seasonal influenza viruses predominant in the patient population have been resistant to AMT or OSC. The development of novel and improved anti-influenza

drugs is still an international need; therefore, it is important to have well characterized influenza virus strains for screening potential inhibitors. For drug candidates with varying MOA, it is important to understand the compound sensitivity to distinct wild type and resistant clinical strains through development of a robust cross-resistance profile. ImQuest has evaluated RBV, AMT, and OSC against a panel of wild-type and drug resistant subtype A and subtype B influenza viruses in order to better understand the replication kinetics and phenotype of each strain. Time of drug addition experiments were performed using wild type and drug-resistant influenza viruses to confirm mechanism of antiviral action. Combination therapy is not yet FDA-approved for the treatment of influenza infection; however, given the prevalence for seasonal drug-resistant virus it would be beneficial to develop therapies inhibiting multiple viral targets for broader antiviral activity and a higher genetic barrier to resistance. Using several approved influenza virus inhibitors and RBV, combination therapy was evaluated to determine potential antiviral synergy using several seasonal strains of influenza virus.

Understanding range of anti-influenza efficacy, cross-resistance, mechanism of antiviral action and how a new inhibitor could potentially be used in combination with approved anti-influenza drugs are crucial for developing a better drug to reduce influenza infection.

46 Identification of a Novel Inhibitor of the HIV-1 Integrase-Ledgf Interaction

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Integrase (IN), the third virally-encoded enzyme required for HIV-1 replication, catalyses the insertion of viral DNA into the host chromatin through a multi-step process that involves 3'-end processing (3-P) and strand-transfer (ST) activity. The integration process is facilitated by a number of host and viral proteins sequestered to form the pre-integration complex (PIC). The proteins that comprise the PIC also represent potential targets for therapeutic intervention; in particular the host protein Lens Epithelium Derived Growth Factor (LEDGF) for which a class of compounds known as LEDGINS have shown to be particularly effective.
In this study we sought to identify novel inhibitors of the HIV-1 IN – LEDGF interaction. Several small molecule compounds, identified though in silico screening of a commercial compound database against the LEDGF binding site on integrase, were biologically evaluated. Of the compounds tested, a naturally occurring statin proved to be most effective at disrupting the protein-protein interaction (IC_{50} = 1.98 ± 1.12μM) as determined through an Alphascreen assay. The statin was found to inhibit viral replication within the MT-4 assay (EC_{50} = 26.24 ± 4.90μM) at non-toxic levels (CC_{50} = 78.17 ± 10.27μM). While the compound demonstrated minimal selectivity in a cell based environment (SI value = 2.98), it yielded no response in an ST activity assay (IC_{50} ≥ 100μM). A novel, useful inhibitor of the IN-LEDGF interaction has therefore been identified for further investigation.

47 Influenza A/NWS/33 (H1N1) Is Uniquely Sensitive to Oseltamivir Treatment in Mice Compared to More Recent Influenza Isolates.

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Oseltamivir is a neuraminidase inhibitor used to treat influenza infections and has been shown to protect mice from a lethal challenge with various influenza strains. A previous study noted that oseltamivir had different efficacies against different influenza strains. When compared to the Influenza A/California/04/2009 (H1N1), AVictoria/3/75 (H3N2) and A/Duck/MN/1525/81 viruses in a lethal mouse model, the Influenza A/NWS/33 (H1N1) virus was more significantly impacted by oseltamivir treatment. Oseltamivir treatment prevented mortality from the A/NWS/33 virus and reduced weight loss and inflammation of the lungs from infection. We examined *in vitro* replication in MDCK cells. Replication curves were not significantly different. In addition, an antiviral assay and neuraminidase inhibition assay were performed to determine the sensitivity of each virus to oseltamivir. Differences were observed but did not explain the *in vivo* observations. Mice were challenged with a

lethal dose of each virus strain and treated with oseltamivir for five days. Mice were sacrificed at various times to ascertain the impact of treatment on lung virus titers, lung lesion scores, lung weights, and mouse body weight. The A/NWS/33 virus was particularly impacted by oseltamivir treatment. At 5 mg/kg/day of oseltamivir, the mice did not demonstrate significant weight loss. Lung titers were significantly lower in mice infected with the A/NWS/33 virus compared to the other viruses. Lung weights were also lower in A/NWS/33-infected mice. Histopathological examination confirmed particular sensitivity of the A/NWS/33 virus to oseltamivir that was observed in lung weights and lung lesion scores. The sensitivity of the A/NWS/33 influenza virus to oseltamivir treatment should be considered when utilizing the virus in an experiment involving neuraminidase inhibitors. This work was Supported by Contract N01-AI-30063 (awarded to Southern Research Institute) from the Virology Branch, DMID, NIAID, NIH.

48 1,2,4-triazoles as Dual-function Inhibitors of HIV-1 Reverse Transcriptase Polymerase and Ribonuclease H Activities

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HIV-1 reverse transcriptase (RT) has two distinct enzymatic activities: RNA/DNA-dependent polymerase activity, and ribonuclease H (RNH) activity. The active sites for these activities locate on one RT subunit and are separated by approximately 40Å, a distance corresponding to 17-18 base pairs of an RNA/DNA duplex. Currently, more than half of the FDA-approved therapeutics against HIV infection target RT polymerase activity; none target RNH activity despite its critical function in HIV replication. Screening studies in our laboratory identified certain 1,2,4-triazole derivatives as potent inhibitors of both the HIV-1 RT polymerase and RNH activities *in vitro* and in cell-based HIV replication assays. Some of the triazoles inhibited RT RNH activity with 10-fold higher potency than for inhibition of RT polymerase activity, yet were unable to inhibit a catalytically active isolated RT RNH domain fragment. Furthermore, mutations in the RT polymerase domain associated with resistance to nonnucleoside RT inhibitors (NNRTIs) resulted in significantly reduced inhibition of RT RNH by triazoles *in vitro* as well as a loss of antiviral activity in cell-based HIV replication assays. Detailed biochemical profiling together with combinational inhibitory assays allowed us to suggest that triazole RNH inhibitors exert their inhibitory activity through binding to the RT polymerase domain, but not the RNH domain. These findings are in agreement with previously published crystal structure of HIV RT in complex with a 1,2,4-triazole compound that showed triazole bound in the HIV-1 RT NNRTI binding pocket. Structural and mechanistic information of how these NNRTI-site binding RNH inhibitors exert their inhibitory activity may prove useful in the design of future novel NNRTIs with dual function inhibition (RT polymerase, RT RNH) *via* binding to a single site on the enzyme. This work was supported in part by grants AI073975, AI077424, and AI100890 from the National Institutes of Health.

49 HCV Polymerase Elongation Complex Formationand Characterization of a New Activity: NTP Mediated Nucleotide Excision

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BACKGROUND: NS5B is the RNA-dependent RNA polymerase responsible for replicating hepatitis C virus (HCV) genomic RNA. Despite more than a decade of work, the formation of a highly active NS5B polymerase RNA complex suitable for mechanistic and structural studies has remained elusive. RESULTS: Through a novel way of optimizing initiation conditions, we were able to convert the majority of NS5B into a functional NS5B-primer-template elongation complex. The elongation complex was very stable, allowing the removal of excess nucleotides and abortive initiation products so that the purified complex was suitable for pre-steady-state kinetic analyses of single nucleotide incorporation. For example, a single CTP could be incorporated with an apparent K_d and k_{pol} values of 39 μM and 16 s^{-1}, respectively, giving a specificity constant of k_{pol}/K_d of 0.41 $\mu M^{-1}s^{-1}$. The elongation complex was highly processive, and allowed the determination of sequence specific variation of nucleotide incorporation kinetics during multiple nucleotide incorporation reactions. We found that in addition to its RNA

synthesis activity, the polymerase elongation complex can use NTPs to excise the terminal nucleotide in nascent RNA. For example, ATP, UTP, or CTP could mediate excision of 3´-terminal CMP to generate the dinucleoside tetraphosphate products Ap_4C, Up_4C, and Cp_4C, respectively. Pre–steady-state kinetic studies showed that the efficiency of NTP-mediated excision was highest with ATP. A chain-terminating inhibitor, 3´deoxy-CMP, could also be excised through this mechanism, suggesting important implications for nucleoside drug potency and resistance. CONCLUSIONS AND SIGNIFICANCE: We established a novel way to generate a highly active elongation complex of the medically important NS5B polymerase for structural and functional studies. We also discovered an unexpected NTP-mediated excision activity catalyzed by NS5B, which has never previously been reported for a viral RNA polymerase.

50 Cocktails of Formulated Short Synthetic shRNAs Show Potent Inhibition of Hepatitis C Virus in HCV-Infected Chimeric Mice

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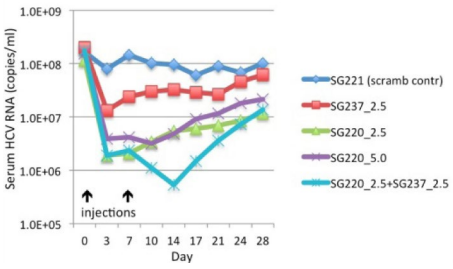
We previously identified 2 short synthetic shRNAs (sshRNAs, SG273 and SG220) that target a conserved sequence within the HCV IRES and potently inhibit HCV IRES-linked reporter expression. To assess *in vivo* activity, SG220 was formulated into lipid nanoparticles (LNP) and injected i.v. into mice whose livers support stable HCV IRES-luciferase expression from a liver-specific promoter. *In vivo* imaging showed a dose-dependent inhibition of luciferase expression (>90% at 2.5 mg/kg sshRNA) with a $t_{1/2}$ for recovery of about 3 weeks. To assess efficacy against HCV infection, uPA-SCID chimeric mice infected with HCV were treated with one or both of the LNP-formulated sshRNAs administered in two doses given i.v. one week apart. At 2.5 mg/kg, SG273 and SG220 produced respectively 1.2 log_{10} and 1.8 log_{10} serum viral load reductions (VLR). The combination of both sshRNAs showed a 2.0 log_{10} VLR following the 1st dose and a further 0.5 log VLR after the 2nd dose. VLR remained >90% at 21 d after the end of dosing. Sequencing of viral RNA amplified from serum after the 21-d follow-up period showed that viral variants mutated in the target region were selected for in the treatment groups but not a control group that received an irrelevant sshRNA. These results demonstrate a direct antiviral activity, with fast and durable HCV suppression via a target-specific RNAi mechanism. This is the first demonstration of *in vivo* efficacy against HCV infection of a synthetic RNAi agent directed against the HCV genome. Partially supported by NIH grant R44AI074256.



51 Non-competitive Inhibition of Hepatitis B Virus Reverse Transcriptase Protein Priming and DNA Synthesis by Clevudine-triphosphate

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All currently approved antiviral drugs for the treatment of chronic Hepatitis B Virus (HBV) infection are nucleos(t)ide reverse transcriptase inhibitors (NRTI), which inhibit the DNA synthesis activity of the HBV polymerase (HP). HP is a unique reverse transcriptase (RT) that has a novel protein priming activity in which HP initiates viral DNA synthesis using itself as a protein primer. We have determined the ability of NRTI-triphosphates (TP) to inhibit HBV protein priming and their mechanisms of action. While entecavir-TP (a guanosine analog) inhibited protein priming initiated specifically with dGTP, clevudine-TP (a thymidine analog) was able to inhibit protein priming independent of the dNTP substrate and without being incorporated into DNA. We next investigated the effect of nucleotide analogs inhibitors on the second stage of protein priming wherein two dAMP nucleotides are added to the initial guanosine nucleotide. The obtained results indicated that clevudine-TP as well as tenofovir-DP strongly

inhibited the second stage of protein priming. Tenofovir was incorporated into the viral DNA primer whereas clevudine-TP inhibited the second stage of priming without being incorporated. Finally, kinetic analyses using the HBV endogenous polymerase assay revealed that clevudine-TP inhibited DNA chain elongation by HP in a non-competitive manner. Thus, clevudine-TP appears to have the unique ability to inhibit HBV RT via an allosteric mechanism, similar to non-nucleoside RT inhibitors.

52 Discovery and SAR Optimization of N-(hetero)aryl-6-(indol-2-yl)pyridine-3-sulfonamides: PTC725, a Potent, Selective and Orally Bioavailable Development Candidate Targeting HCV NS4B

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A novel series of N-heteroaryl-6-(indol-2-yl)pyridine-3-sulfonamides that target the HCV NS4B to inhibit viral RNA replication has been identified through extensive SAR development. Medicinal chemistry efforts were focused primarily on identifying optimal heteroaryl moieties at the indole N-1 and to define the optimal substitution pattern on the indole benzenoid ring to limit the potential for oxidative metabolism *in vivo*. The SAR investigation culminated in identifying compounds with a favorable balance of replicon potency, selectivity and DMPK properties. Several compounds were evaluated for their potential to induce drug-drug interactions and PTC725 was selected for further evaluation. PTC725 exhibits potent activity against the HCV 1b replicon (EC_{50} = 2 nM) with more than 5,000-fold selectivity with respect to cellular GAPDH RNA. PTC725 has a favorable pharmacokinetic profile in rats, dogs, and monkeys with oral bioavailabilities of 62%, 78% and 18%, respectively, and has favorable tissue distribution, with a liver to plasma ratio in rats of 25. PTC725 has as excellent safety profile with no significant toxicity observed at doses up to 2000 mg/kg/day in a 14-day pilot rat safety study making it a good candidate for preclinical development.

53 Chemical Optimization of a Novel Class of 6-(indol-2-yl)pyridine-3-sulfonamides targeting HCV NS4B: Potent and Orally Bioavailable Compounds with an Improved ADMET Profile

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A series of 6-(indol-2-yl)pyridine-3-sulfonamides that target the HCV NS4B to inhibit HCV RNA replication has been identified. Extensive optimization efforts have been performed to improve the ADMET profile of these compounds with two primary objectives: 1) identify a suitable alternative to the 1,3-difluoropropany-2-yl sulfonamide moiety in order to eliminate the putative formation of 1,3-difluoroacetone which could form upon oxidative dealkylation *in vivo*; and 2) reduce the levels of glutathione conjugates formed from reactive indole metabolites *in vivo*. Sulfonamide moieties containing multi-fluorinated alkyl groups modulating the electronic properties of the sulfonamide group were evaluated. Several (*S*)-N-(1,1,1-trifluoropropan-2-yl) sulfonamide analogs with excellent potency and selectivity against the HCV genotype 1b replicon were identified. We also evaluated several substituent combinations on the indole benzenoid ring to reduce the potential for oxidative metabolism *in vivo*. PTC-971, obtained from the addition of a 5-F substituent successfully resulted in reduced levels of glutathione adducts generated *in vivo*. This compound exhibits potent activity against the cell-based HCV 1b replicon (EC_{50} = 7 nM) with more than 1,300-fold selectivity with respect to cellular GAPDH RNA. De novo selection of HCV replicon resistance to PTC-971 resulted in three major amino acid substitutions in NS4B. PTC-971 has a favorable pharmacokinetic profile in rats and monkeys and an excellent safety profile with no significant toxicity observed in rats at doses up to 2000 mg/kg/day in a 14-day pilot safety study.

54 Antiviral Activity of Benzimidazol Derivatives Against Influenza A Virus

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Influenza remains a poorly controlled infection causing annual epidemics and pandemics and at the same time the number of effective flu drugs is limited and besides their wide application leads to an increase in resistant strains. This fact highlights the need to develop new tools against the flu. In recent years, numerous studies have been conducted regarding antiviral properties of substances of natural origin. From another hand the mostly used in clinical practice are compounds of synthetic origin. Particular attention should be given to derivatives of benzimidazole (BI), because those substances have a wide spectrum of biological activity. We have tested 27 derivatives of BI, all were synthesized in Southern Federal University. Pandemic influenza viruses A/California/7/09 (H1N1) and A/Swine/1976/31 were grown in MDCK cells in presence of compounds. After 48 hours of incubation virus yield was evaluated in hemagglutination test. The toxicity of compounds was tested in microtetrazolium test (MTT). Based on the results obtained, the 50% cytotoxicity dose (CTD_{50}), 50% effective dose (ED_{50}) and their ratio – selective index (SI) were calculated. The results obtained demonstrated that 15 (55%) studied compounds of BI derivatives had SI more then 10 (10-41.5) against A/California/7/09 and 4 (15%) of studied compounds had SI more than 10 (19.9-167) against A/Swine/1976/31. A protective effect of the BI derivative was shown against A/California/7/09 on the model of lethal influenza virus pneumonia in white mice. Protection index for this substance at a dose of the virus 1 LD_{50} was 75%. In general, derivatives of BI showed relatively high activity against pandemic influenza and can therefore be recommended for further development for prevention and/or treatment of influenza.

55 Evaluation of Candidate Topical Microbicides in Pharmacokinetic and Pharmacodynamic *In Vitro* Models to Predict the Necessary Concentration Required to Prevent HIV Infection

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The identification and subsequent development of a successful microbicide product to prevent the transmission of sexually transmitted organisms is dependent on the robustness of the efficacy and safety algorithms that are used to advance products. Preclinical and clinical experiences have driven the natural evolution of these algorithms over time and it is understood that these will continue to change in order to adapt to the evolving field. It is well accepted that for a microbicide to be successful it must be at the right place, at the right time, and at the right concentration. *In vitro* pharmacokinetic models have been developed to assess the concentration of a microbicide that is able to permeate through representative cell monolayers to the tissues where the microbicide must accumulate in order to be effective. We have utilized these *in vitro* models, as well as *ex vivo* models including ectocervical and colorectal explants as a means to quantify the required concentration of a microbicide to prevent the transmission of HIV in target cells and tissues. Our data suggest that the candidate microbicide IQP-0528 rapidly penetrates through epithelial cells and explant tissues and achieves an inhibitory concentration. Conversely, the approved antiretroviral Tenofovir was found to be significantly slower in penetrating through epithelial cell monolayers and accumulating at the site of infection. Our data serves to correlate the "sterilizing" concentration of products as determined in the microbicide transmission and sterilization assay (MTSA) to that of the microbicides that have been shown to penetrate cell monolayers *in vitro,* plus those concentrations necessary to protect cervical and rectal explants from infection for these two compounds and other candidate microbicides representing different classes of anti-HIV inhibitors. We believe these assays will better predict the required protective concentration of a microbicide in target cells and tissues to prevent infection and will better inform animal modeling and human clinical trial dosing regimens.

56 Amantadine Analogs that Inhibit MDCK Cell Infection by H1N1 2009 Influenza A Containing M2(S31N).

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The M2 mutation of Ser31 to Asn has become dominant globally in human infections by influenza A since 2005, rendering the virus resistant to the FDA-approved prophylactics, amantadine and rimantadine. Attempts to identify alternative M2 blockers have been futile until last year. Here we report that infection of cultured Madin-Darby canine kidney cells by Influenza A/California/04/2009 (H1N1 swine flu), which bears the S31N mutation, is not blocked effectively by amantadine or rimantadine, but is effectively blocked by 16 amantadine variants. Influenza A (H3N2) Victoria/3/75 was effectively blocked by amantadine (3 μM EC50) and rimantadine, as expected. EC50s for the novel drugs against the S31N-containing virus range from 0.8-36 μM. 8 of these 16 compounds were previously reported to block influenza A with wild type M2 and are therefore effective against both strains. Liposome assays indicate that proton transport by M2 (22-62, S31N) is blocked by the drugs and drug binding was detected in M2 (22-46, S31N) with NMR and CD. The H1N1 virus was tested *in vitro* for development of drug resistance against one of the compounds and compared to drug resistance development in an M2 wild-type virus exposed to amantadine. The test drug selected resistance mutants considerably later in serial passage than occurred in the amantadine-wild type control.Toxicity in mice is low for three representative compounds. The success with the 8 novel syntheses suggests that a moderately sized alkyl group attached at C2 to 2-amino-adamantane is effective against M2 (S31N) with attenuated resistance formation.

57 Evaluation of RNA-knockdown Strategies for Modulation of Influenza Virus Matrix Gene Activity in Mammalian Cell Line

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Hidden formatting deleted. Delete this text! justifyline-height:150%"> Background: DNAzymes (Dz), derived by *in vitro* selection processes, is one such discovery that has potential for selective gene silencing; thus we aimed to target the M2 gene of influenza A virus to down-regulate its replication in MDCK cells. Materials and Methods: Several 10-23 DNAzymes were designed and analyzed for their ability to specifically cleave the M2 gene of influenza A virus. The Dz that worked best was further standardized with $MgCl_2$ gradient to achieve the best results. The same concentrations of Dz were also transfected with the whole virus (Influenza A/PR/8/34) to study the inhibition of replication. RT-PCR and Real-time RT-PCR assays followed by western blot analysis were performed to detect the inhibition in the expression of M2 gene. Results: We observed that the Mg^{2+}-dependent sequence specific cleavage of M2 RNA was achieved up to 80% in a dose-dependent manner. The transfection of the MDCK cells with the Dz also reduced the cytopathic effect caused by A/PR/8/34 (H1N1) and this antiviral effect persisted for almost 48 hours. The western blot revealed significant down-regulation in the M2 protein thereby reducing the virus replication in host cells. Conclusion: The designed Dz significantly down-regulated the virus replication in MDCK cells. Since there are very few studies done on applications of DNAzymes against influenza viruses, there is good prospect of its therapeutic use to protect from the lethal effects of influenza A viruses.

58 QSAR Modeling of Anti-Influenza (A/H3N2) Activity

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Influenza spreads around the world in seasonal epidemics, resulting in the death of up to half of a million people every year. Since vaccination and existing antivirals cannot guarantee a complete protection against influenza,

battling this virus remains important health care issue that requires the design and development of new drugs. Application of computational methods shortens the development time and reduces costs of antiviral drug research. The goal of this study is to design novel selective agents by means of Quantitative Structure-Activity Relationship QSAR modeling of antiviral activity of various compounds against influenza. The dataset consisted of 97 diverse chemicals. Antiviral activity against influenza strain A/Hong Kong/1/68 H3N2 was assessed as effect on virus-induced cytopathogenicity in MDCK cells (lgEC_{50}, M) Thorough investigation of the relationship between the antiviral activity and the structure of investigated compounds was carried out using Hierarchic QSAR Technology (HiT QSAR) based on Simplex representation of molecular structure (SiRMS). Prior to development of QSAR analysis, the dataset was carefully curated from both chemical and biological perspectives. Predictive power of developed models was estimated by five-fold external cross-validation as high as $R^2 = 0.76$. Molecular fragments effecting the variation of anti-influenza activity and their average relative influences were determined. For instance, the presence of 2-iminomethylphenol fragment was shown to increase the antiviral activity, whereas aminoethylene decreased it. More than 100 novel anti-influenza agents were computationally designed and predicted using developed models. Five of them were recommended to synthetic and biological experiments. KEY WORDS: *Influenza A/H3N2, QSAR, computer-assisted drug design.*

59 A Michael-acceptor-type 3C Protease Inhibitor With Broad-spectrum Anti-rhinoviral Activity

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SG85, a peptidic α,β-unsaturated ethyl ester, was developed during a structure-based design effort of Michael acceptor inhibitors of the enterovirus 3C protease (Tan *et al*, J Virol, 2013). We now demonstrate that SG85 efficiently inhibits a panel of different rhinovirus serotypes, including both rhinovirus A and rhinovirus B strains as well as minor and major receptor group strains, with EC_{50} values ranging between 5 and 200 nM. A drug-resistant human rhinovirus 14 (hRV14) variant was selected that exhibited >4-fold less susceptibility to SG85 than the wild-type virus. Genotyping of the drug-resistant hRV14 revealed a two-amino-acid mutation in the 3C protease coding region (S127G and T143A). hRV14 resistant against rupintrivir (a well-known enterovirus 3C inhibitor) also carries a mutation at residue T143 of 3C (Binford *et al*, Antimicrob Agents Chemother, 2007). Reverse engineering of the mutations into an infectious clone of hRV14 is ongoing, in order to assess the impact of the mutations on the resistant phenotype.

60 T-cell Mediated Immunity Activation in HIV/HCV Coinfected Patients with AIDS

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AIM OF STUDY: to evaluate T-cell mediated immunity activation in HIV/HCV coinfected patients with AIDS

MATERIAL AND METHODS. The HIV/HCV coinfected patients included in the study were divided into two groups. Group 1 – 40 patients without AIDS, mean age 33,9±5,8 yrs, males – 32 (80%), females – 8 (20%). Group 2 – 11 patients with AIDS, mean age 34,8±6,5 yrs, males – 7 (63,6%), females – 3 (27,2%). AIDS was established if level of CD4+ T-lymphocytes was less than 200 cells/μL and/or if patent had the fourth stage of HIV-infection according to WHO classification, 2006. The cells were analyzed using «FACSCalibur» flow cytometer («Becton Dickenson», USA). The monoclonal antibodies «Becton Dickenson», USA were used. Plasma viral load (VL) of RNA HIV were detected using Ampisens monitor (Russia).Data are presented in median (%/cells/μL) (range).

RESULTS. Percentage of CD3+/HLA-DR (30.5 (10.8- 59.8) vs. 48.4 (34.7- 63.8), respectively, $p<0,01$) and CD3+CD8+/HLA-DR (30.0 (12.8-59.0) vs. 44.1 (32.3 - 60.3) respectively, $p=0,02$) was lower in non AIDS patients in comparison with AIDS patients.Percentage of CD4+/HLA-DR did not significantly differ in the compared groups

but absolute CD4+/HLA-DR index in AIDS group was significantly lower in comparison with non AIDS (51.5 (10.0 -75.7) vs. 79.9 (48.4 - 274.5), respectively, p=0,004). Percentage of CD4+/CD25+ cells (1.0 (0.3 – 1.7) vs. 2.3 (0.7 – 6.7), respectively, p=0,004) and absolute number of CD4+/CD25+ cells (10.6 (1.0 – 34.2) vs. 43.4 (13.8 – 224.0), respectively, p=0,002) was significantly lower in AIDS in comparison with non AIDS. Negative correlation of CD4+/ CD25+ index with HIV viral load (Spearman's R correlation = – 0,75, p<0,0001) was detected.

CONCLUSIONS. In natural disease course of HIV/HCV coinfection immunity activation of cytotoxic blood T-lymphocytes was more evident at AIDS stage. Activation of CD4+ T-lymphocytes in HIV/HCV-infected patients was decreased at AIDS stage. AIDS development led to decreasing content of CD4+/CD25+ blood lymphocytes which had an opposite correlation with serum HIV viral load.

61 Differential Time-dependent Activities of Small Molecule Inhibitors of Respiratory Syncytial Virus (RSV) Targeting Distinct Entry And Post-Entry Steps in the Virus Replication Cycle

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Human respiratory syncytial virus (RSV) is a single stranded, negative sense RNA virus *that* causes acute, occasionally fatal, lower respiratory illness in infants, the elderly, and immunocompromised patients. The lack of safe and effective vaccines has led to increased research efforts towards the development of therapeutic anti-RSV drugs. To better understand the biological profile of an optimal RSV therapy, we studied the *in vitro* transcription and replication kinetics during a single cycle viral infection in Hep2 cells, in the presence and absence of a panel of established, small molecule RSV inhibitors. This panel included the entry inhibitor **TMC-353121** (Bonfanti *et al*, J Med Chem. 2008) and post-entry inhibitors, **ribavirin** (Sidwell *et al*, Science 1972), **YM-53403** (Sudo *et al*, Antiviral Res 2005), **BI-D** (Liuzzi *et al*, J Virol 2005) and **RSV-604** (Chapman *et al*, Antimicrob Agents Chemother 2007). Unique time of compound addition profiles were observed for each class of inhibitors. The entry inhibitor TMC-353121, targeting the viral F protein, rapidly lost activity within the first hour of the infection cycle. RSV-604, which targets an unknown mechanism susceptible to resistance mutations in the RSV N gene, showed a gradual loss of antiviral activity following 3 hours post-infection, indicative of blocking an iterative process starting early in the replication cycle. The non-catalytic RNA polymerase inhibitors YM-53403 and BI-D, as well as ribavirin, retained their activity irrespective of the time of compound addition, and dramatically decreased the steady-state levels of intracellular viral mRNA and genomic RNA. The retention of antiviral effects during the late stages of the RSV replication cycle was correlated with the ability of the tested compounds to inhibit the RSV sub-genomic replicon. Our data suggests that the established potent activity and animal model efficacy of RSV fusion inhibitors may be complemented by the extended action and intracellular viral RNA clearance profile of inhibitors with post-entry mechanisms.

62 Mechanisms of Hypersusceptibility to EFdA-TP by NRTI- and NNRTI-resistant HIV RTs

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The K65R substitution in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) is the major resistance mutation selected in patients who receive treatment that includes the nucleotide analog tenofovir disoproxil fumarate (TDF). Y181C is one of the most common RT mutations which arises early upon administration of most non-nucleoside RT inhibitors (NNRTIs). 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) is a nucleoside analog that unlike all approved anti-HIV-1 nucleoside RT inhibitors (NRTIs) retains a 3'-hydroxyl group and has remarkable potency against wild-type (WT) and drug-resistant HIVs. EFdA acts primarily as a chain terminator by blocking translocation following its incorporation into the nascent DNA chain. Here we report that the K65R mutation and to a lower level the Y181C HIV-1 RT mutation cause hypersusceptibility to EFdA. Specifically, in single replication cycle experiments we found that EFdA blocks WT HIV ten times more efficiently

than TDF. Under the same conditions K65R HIV was inhibited over 70 times more efficiently by EFdA than TDF. Enzymatic studies with WT and K65R RT helped us determine the molecular mechanism of this hypersensitivity. We found that this change causes minor changes in the efficiency of EFdA incorporation with respect to the natural dATP substrate and also on the efficiency of RT translocation following incorporation of the inhibitor into the DNA primer. However, a significant decrease in the excision efficiency of EFdA-MP from the 3' primer terminus appears to be the primary cause of increased susceptibility to the inhibitor. Notably, the effects of the mutation are DNA-sequence dependent. Similar results were obtained for the Y181C mutation. In conclusion, we have elucidated the mechanism of K65R and Y181C HIV hypersusceptibility to EFdA. Our findings highlight the potential of EFdA to improve combination strategies against TDF-resistant and NNRTI-resistant HIV-1 strains.

63 Ten Years Experience Observing Generation and Preservation of Specific Antibodies Against Measles in Children Born From HIV Positive Mothers with Confirmed HIV Infection

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Starting from 2002 conducting surveillance for specific antibodies against measles by vaccinating 41 children born from HIV positive women with a confirmed diagnosis of HIV infection. Vaccination was carried out if absolute CD4 + cell count was at least 25% of the age norm. Measles vaccines were used: Russian monovalent measles divalent vaccine measles and mumps, vaccine Priorix (GlaxoSmithKline, Belgium) and MMR-II (MSD, USA). 68,7% of children were seronegative at day 14th. At day 45th after vaccination 24,1% of children had protective antibody titers and 62,1% had minimal protective titers. 5 children with HIV infection which stay seronegative after vaccination, was re-immunized at 6 months later. Already on day 14th, measles antibody titer was 5 times higher ($p < 0.01$), and to 45th day significantly higher than those vaccinated only once ($p < 0.05$). At age of six years, 6 children in study group were revaccinated against measles. All the children were seronegative prior to vaccination. At day 14th only one child remained seronegative, others have developed protective immune response. At 45th day all children had protective immunity. 2 children received a booster dose of measles vaccine at day 14th after vaccination, develop protective titers. Revaccination after 5 years were examined in 16 children. Of these, 43,75% were seronegative and only 18,75% of children had a high level of protective antibodies. Thus, the booster vaccination within 6 months after the first provides the immunological efficacy of measles vaccination in HIV-positive children. Even with the timely conduct of revaccination, in case of contact with measles, all HIV-positive children's should be tested for measles antibody titer and if absent or in case of inability to conduct a survey, specific immunoglobulin should be given within 72 hours after exposure at a dose of 0,5 ml/kg body weight (not more than 15 ml).

64 Activation of the Antiviral Agent T-705 (favipiravir) by Hypoxanthine Guanine Phosphoribosyltransferase

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6-Fluoro-3-hydroxy-2-pyrazinecarboxamide (T-705) is a novel antiviral compound with broad activity against influenza virus and other RNA viruses. Its active metabolite, T-705-ribose-5'-triphosphate, inhibits the influenza virus RNA polymerase by competing with GTP [Furuta et al., Antimicrob Agents Chemother. 2005]. The enzymes responsible for activation of T-705 remain to be identified. We here report that T-705 is directly converted into its ribose-5'-monophosphate metabolite by hypoxanthine guanine phosphoribosyl transferase (HGPRT). In a virus yield assay in MDCK cells, T-705 and 3-hydroxy-2-pyrazinamide (the analogue of T-705 lacking the 6-fluoro atom) displayed antiviral EC_{90} values of 6 and 1 μM, respectively. Their activity was totally lost ($EC_{90} > 500$ μM) in HGPRT-deficient MDCK cells. In HEK293T cells undergoing siRNA-mediated gene knockdown followed

by the viral ribonucleoprotein reconstitution assay, the inhibitory effect of T-705 on influenza virus was 8-fold reduced upon HGPRT gene knockdown. In contrast, gene knockdown for adenine phosphoribosyltransferase or nicotinamide phosphoribosyltransferase did not change the activity of T-705. Enzymatic assays revealed that T-705 and 3-hydroxy-2-pyrazinamide are very weak substrates for human HGPRT (K_m values: 9.6 and 3.7 mM, respectively, *versus* 3.6 and 5.4 μM for hypoxanthine and guanine, respectively). The analogue 2-pyrazinecarboxamide was not converted by HGPRT. Our data indicate that ribophosphorylation and antiviral activity of these carboxamide compounds requires the presence of the 3-hydroxyl but not the 6-fluoro function. Since conversion of T-705 by HGPRT appears inefficient and rate-limiting, design of structural derivatives with an improved activation profile may help to increase the antiviral potency of these carboxamide analogues. Relevant information should come from our ongoing studies to reveal the binding mode of T-705 and 3-hydroxy-2-pyrazinamide within the catalytic site of HGPRT.

65 Galectin-3 Promotes HIV-1 Expression in Latently Infected Cells Through NF-κB Activation

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Antiretroviral therapy (ART) has achieved successful reduction of plasma viral load in HIV-1-infected patients below the undetectable level. However, the current ART cannot eradicate the virus from patients because latently infected cells cannot be eradicated from their bodies. The latently infected cells exist for a long period of time in patients as viral reservoirs, which lead to viral rebound in case of treatment interruption. The mechanism of HIV-1 expression from latently infected cells, especially cellular factors involved in this process, has not been fully understood yet. Galectin-3, a member of the lectin family binding to β-galactoside, is widely expressed on various cells including macrophages and activated T cells. Galectin-3 regulates proliferation, differentiation, and apoptosis of the cells and plays an important pro-inflammatory role. In this study, we have demonstrated that Galectin-3 promotes HIV-1 expression in latently infected cells. When the latently infected cell line OM-10.1 was treated with TNF-α, along with HIV-1 expression, Galectin-3 expression was up-regulated at both mRNA and protein levels in a dose dependent manner. Knockdown of Galectin-3 expression by iRNA resulted in the suppression of HIV-1 production in TNF-α-treated OM-10.1 cells. Furthermore, the suppression of Galectin-3 reduced the TNF-α-induced nuclear translocation of NF-κB in the cells. These results suggested that Galectin-3 promotes HIV-1 expression in latently infected cells through NF-κB activation and may be a potential target for inhibition of HIV-1 replication by small-molecule compounds.

66 Surveillance for Neuraminidase Inhibitor Susceptibility of Influenza Viruses in 2011–2012: Application of New WHO Criteria

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Surveillance for susceptibility to neuraminidase (NA) inhibitors (NAIs) is critical as these drugs are the only available therapeutic option for influenza infections due to widespread resistance to M2 blockers. Since cell culture-based assays are unreliable, NAI susceptibility is primarily assessed in NA enzyme inhibition (NI) assays, supplemented by NA sequencing. However, there are no established criteria for IC_{50}s (concentration of drug inhibiting virus NA activity by 50%) indicative of clinically relevant resistance. Moreover, lack of NI assay standardization results in variable IC_{50}s, creating challenges in sharing and interpreting IC_{50} data within the WHO surveillance system. Thus, the WHO working group on influenza antiviral susceptibility (WHO-AVWG) developed new criteria for interpreting IC_{50} data. We applied these criteria on IC_{50}s for two FDA-approved NAIs, oseltamivir and zanamivir, and two other NAIs, peramivir and laninamivir. All A(H1N1)pdm09 viruses (n=1088) collected in the 2011-2012 season exhibited 'normal' inhibition by the NAIs, except for 28 (3%) viruses with H275Y substitution which exhibited 'highly reduced' inhibition by oseltamivir and peramivir, and two with 'highly reduced' inhibition by zanamivir due to cell-culture selected changes. All A(H3N2) viruses (n=2216) exhibited 'normal' inhibition,

except for one with the R292R/K change that showed 'highly reduced' and 'reduced' inhibition by oseltamivir and zanamivir, respectively, and another with 'reduced' inhibition by zanamivir due to cell-culture selected changes. All B viruses (n=1325) exhibited 'normal' inhibition, except for an isolate (A200A/T), with 'reduced' inhibition by oseltamivir, zanamivir and peramivir, and another (G140G/E and H273N) with 'reduced' inhibition by zanamivir. The WHO-AVWG criteria, coupled with NA sequencing, effectively detected viruses carrying markers of NAI resistance (e.g. H275Y and R292K), as well as changes resulting from virus propagation (e.g. Q136K). These criteria will be useful in harmonizing NI assay data and establishing laboratory correlates of clinically relevant resistance.

67 Simultaneous Detection of Dengue Virus NS1 Antigen, IgM and IgG Antibodies in Febrile Cases in Ibadan, Nigeria

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The absence of specific treatment and vaccine has made it imperative for surveillance data collection to aid in the control and preventive programmes.The epidemiology of dengue transmission and incidence in African countries including Nigeria are poorly defined, leading to a great under recognition and underreporting, even in areas where there is a high level of public health awareness and diagnostic capacity. Often many patients with fever are designated as having fever of unknown origin or malaria and remain without a diagnosis even if they fail to respond to antimalaria drugs.Therefore cases of dengue in Africa are frequently reported among travelers while the prevailing practices in many countries coupled with the non-availability of diagnostics has created a great potential for misdiagnosing dengue locally.This study therefore investigated the prevalence of dengue virus infection among clinically febrile cases presenting at government hospitals in Ibadan, the third largest city in Nigeria. One hundred and eighty-eight (188) sera samples collected from cases were screened for evidence of dengue virus infection using NS1 antigen, IgM and IgG antibodies detection assays.A two-step sandwich immunoassay, MAC ELISA and indirect ELISA(DAI, Calabasas, USA) were used for the identification of NS1 and the antibodies respectively. Serological evidence for dengue virus infection was observed in 33(17.6%), 61(32.4%) and 76(40.4%) samples using the NS1, IgM and IgG detection assays. The NS1 positive samples included 17 and 15 that otherwise would have been missed because they were IgM and IgG negative. Seventeen samples (9%) had triple positivity (NS1, IgM and IgG) while 44 and 61 negative for NS1 antigen were positive for IgM and IgG respectively.The triple immunoassays complemented each other in that both the early and latter phases of primary and secondary infections were diagnosed.The study establishes the public health role of dengue in Ibadan as well as providing a background data on the prevalence of dengue fever among febrile cases in Nigeria.However it is strongly advocated that dengue fever is included in the differential diagnosis of acute febrile conditions in Nigeria.

68 SPIRIT: Switching to Rilpivirine/Emtricitabine/Tenofovir DF Single-Tablet Regimen from Boosted Protease Inhibitor Maintains HIV Suppression at Week 48

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ARV regimen simplification improves quality of life and medication adherence while reducing the risk of HIV virologic failure and long-term drug-related toxicities. Rilpivirine/ Emtricitabine/Tenofovir DF (RPV/FTC/TDF) is a well-tolerated and efficacious once daily single-tablet regimen (STR) treatment option. Here we report the Week (W) 48 safety and efficacy results of SPIRIT, the first study to evaluate switching from boosted protease inhibitor (PI+RTV+2NRTI)-based regimens to the simplified STR RPV/FTC/TDF. SPIRIT is a phase 3b, open-label, international, 48-week study to evaluate the safety and efficacy of switching from PI+RTV+2NRTI regimens to RPV/FTC/TDF in virologically-suppressed HIV-1 infected subjects. Subjects were randomized 2:1 to switch to RPV/FTC/TDF at baseline (BL) or maintain their current PI+RTV+2NRTI regimen with a delayed switch to

RPV/FTC/TDF at W24. The primary endpoint was non-inferiority (12% margin) of RPV/FTC/TDF relative to PI+RTV+2NRTI regimens in maintaining virologic suppression (VS plasma HIV-1 RNA <50 copies/mL) at W24 by FDA snapshot analysis. A total of 476 subjects were randomized and received at least 1 dose of study drug (RPV/FTC/TDF, n=317 PI+RTV+2NRTI, n=159). BL characteristics were similar across treatment arms. The primary endpoint of non-inferiority at W24 was met (93.7% RPV/FTC/TDF vs 89.9% PI+RTV+2NRTI, difference 3.8%, 95% CI[-1.6 to 9.1]). Through W48, 89.3% of subjects switching to RPV/FTC/TDF at BL maintained VS. The rate of VS at W48 for the 152 subjects who switched to RPV/FTC/TDF at W24 (92.1%) was comparable to the rate of VS at W24 for those who switched to RPV/FTC/TDF at BL. In the W48 analysis of SPIRIT, the first study to evaluate switching to RPV/FTC/TDF STR from a PI+RTV+2NRTI based regimen in virologically-suppressed, HIV-1-infected subjects, VS was maintained regardless of whether subjects switched to RPV/FTC/TDF at BL or at W24.

69 *In Vitro* Efficacy Profiling of Protease Inhibitors in Genotype 4a HCV Replicons

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BACKGROUND & AIMS: Genotype 4a (GT4a) HCV is prevalent in the Middle East, North Africa and increasingly found in Europe. Here we report antiviral activity profiling for a panel of NS3 protease inhibitors (PIs) against GT4a using a novel replicon system.

MATERIALS & METHODS: A robust GT4a (ED43 strain) subgenomic replicon was established in Huh7-1C cells. NS3 chimeric replicons were constructed by cloning various GT4a NS3 protease domains into a GT1b replicon. NS3 polymorphisms were identified by aligning GT4a sequences from the EU HCV database. Polymorphisms were introduced into a wild-type GT4a replicon by site-directed mutagenesis. Antiviral potency was determined in 3-day replicon assays.

RESULTS: The PIs GS-9451, TMC-435 and MK-5172, showed minimal EC_{50} differences between GT4a and GT1b (<2-fold shifts, Table 1). Telaprevir was 4-fold less active against GT4a compared to GT1b, supporting its reduced efficacy in GT4a patients. GT4a polymorphisms found in >5% of sequences (T54S, T122S, I132L, I132V, V170I) did not substantially change PI susceptibility (<3-fold EC_{50} changes compared to the parent GT4a replicon). To further evaluate polymorphisms, the NS3 domain from four clinical isolates was cloned into aGT4a chimeric NS3 replicon; these isolates had PI susceptibility similar to the parental GT4a replicon. Despite its higher potency, mutations conferring >100-fold resistance (e.g. D168Y and A156T) were readily selected in GT4a replicons by the third-generation PI MK-5172.

CONCLUSIONS: With the exception of telaprevir, the clinical stage PIs tested here had similar potency against GT4a and GT1b. PIs retain potency against major NS3 protease polymorphisms in GT4a, but have significant weaker activity against *in vitro* selected resistant mutants.

Table-1. Antiviral Activity of PIs Against GT4a and GT1b

Compound	EC_{50} (nM)		
	GT1b (subgenomic)	GT4a (subgenomic)	GT4aPr/1b (NS3 chimeric)
GS-9451	20.4	21.4	10
TMC-435	4.4	5.3	2
MK-5172	0.8	1.5	1.1
Telaprevir	656	2578	2379

70 Targeting the Organic Anion Transporter-3 (OAT3) with Probenecid as a Novel Anti-Influenza A Virus Strategy

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Influenza A virus infection is a major global health concern causing significant mortality, morbidity, and economic losses. Antiviral chemotherapeutics that target influenza A virus components are available for treatment of influenza A virus infections; however, rapid emergence of numerous drug resistant strains has been reported due to its high rate of mutations and reassortments which drive selection for escape mutants. Consequently, there is an immense need to identify novel anti-influenza A drug targets and for the subsequent development of corresponding chemotherapeutic agents. In this study, we utilized an siRNA screen of host drug target genes to identify novel targets for anti-influenza A therapy. We identified a host organic anion transporter, OAT3, that is important to support influenza A virus infection. We demonstrated that probenecid, a prototypical chemical inhibitor of organic anion transporters, was effective in limiting influenza A virus infection *in vitro* and *in vivo*. Furthermore, we identified that probenecid acts to inhibit influenza A virus attachment by downregulating surface expression of its receptor sialic acid. Probenecid is currently available for clinical treatment of gout and other hyperuricemic disorders and has been extensively studied for its pharmacokinetics and safety. Thus, probenecid is an excellent candidate for repositioning as a novel anti-influenza A chemotherapeutic, significantly reducing both cost and time for its development from bench to bedside use.

71 KPT-335, a Novel Inhibitor of Nuclear Export (SINE), Reduces Influenza A Virus Replication *in vitro* and *in vivo*

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Influenza is a major global health concern causing significant mortality, morbidity, and economic losses. Chemotherapeutics that target influenza are available; however, rapid emergence of drug resistant strains is common. Therapeutic targeting of host proteins hijacked by influenza to aid its replication can be used as an antiviral strategy to reduce the development of drug resistance. Nuclear export of influenza ribonucleoprotein (vRNP) has been shown to be mediated by host exportin CRM1 (XPO1/exportin1) interaction with nuclear export signal (NES)-containing viral proteins NEP and M1 tethered to vRNP. Using siRNA screening of a human drug target library, we identified CRM1 as a host pro-influenza factor. We recently demonstrated that CRM1 silencing results in reduced influenza replication. CRM1 inhibitor, leptomycin B (LMB), has been previously shown to limit influenza replication *in vitro*; however, LMB is toxic *in vivo* rendering it unsuitable for therapeutic use. In this study, we show that an orally available small molecule, KPT-335, potently and selectively inhibits CRM1, and can be used to inhibit influenza virus replication *in vitro* and *in vivo* with minimal toxicity. Prophylactic administration of 20mg/kg KPT-335 for either 2 or 3 days prior to infection with influenza A/California/04/09(H1N1) or A/Philippines/2/82(H3N2) provided protection and was associated with a significant decrease in viral lung titers at 5 days post-infection. Therapeutic administration of KPT-335 at days 1, 3, or 4, post-infection also was efficacious and reduced lung viral titers. These studies suggest that KPT-335 acts as a novel anti-influenza A therapeutic agent.

72 The Discovery and Characterization of Novel Bioactive Small Molecules Targeting the Priming Complex of HIV-1

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The ability of HIV to evolve in response to therapeutic pressure creates a perpetual need for new therapeutics. The next generation of therapeutics will likely target viral functions not yet exploited, such as the HIV viral priming complex. This complex is essential for activation of the reverse transcriptase enzyme and represents a therapeutic target that involves a host factor. HIV selects only human $tRNA^{Lys3}$ for this primer. Following extensive transformation of the native conformation, HIV modifies the anticodon stem loop (ASL) into a platform for

creating this primer complex. In addition to the 18 basepair duplex formed between the viral genome and the 5'end of human $tRNA^{Lys3}$ at the viral primer binding site, there is second region of interaction. This region has been shown to be essential for viral replication in both deletion and antisense characterization studies which validate this interaction as a target for therapeutic intervention. To discover small molecules therapeutics, a screening assay design was created using synthetic oligonucleotides, as mimic of the priming complex, integrated into an AlphaScreen™ discovery detection platform. Using this assay over 300,000 chemically diverse small molecules identified over 1000 hits. After confirming true hits in reanalysis with the screen, determination of bioactivity was made by testing over 250 compounds for inhibition of viral replication in a PBMC assay. This secondary screen identified a collection of at least 40 bioactive hits, among diverse chemical families, with a therapeutic index range of 4-63. Biochemical and structural characterization by NMR have confirmed specific interactions between the bioactive hits and the complex and provided atomic level models of the RNA/RNA/small molecule complexes. The structural studies and additional SAR studies that have found bioactive analogs provide additional validation of a screen for novel therapeutic discovery and future intervention.

73 Are Antiviral Drugs Against Influenza Targeting Cell Signaling Pathways Inherently Toxic?

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Influenza virus interacts with host cell proteins and exploits a variety of cellular pathways for its own benefit. This renders these pathways as targets for new therapeutic intervention. Compounds inhibiting these pathways are viewed as attractive candidates for the development of targeted therapies of cancer. Few of them are already licensed for anti-cancer therapy. Nevertheless, there is a common assumption that any drug developed for the treatment of cancer must be inherently toxic, so that its use for antiviral therapy will necessarily be limited to the most severe infections or these compounds are not suitable as antivirals at all. In our previous work we were able to demonstrate that influenza virus replication is dependent on Raf/MEK/ERK and NFkappaB signaling pathway activity and that inhibition of these pathways lead to reduction in viral titer. In the present work we present evidence from pre-clinical approaches that signaling inhibitors developed for cancer therapy and also show antiviral properties against influenza might not necessarily be inherently toxic. We could show that MEK-inhibitor treatment of H1N1pdm09 infected C57BL/6 mice is effective in reducing virus titer in the lung using concentrations below the once required reducing growth of cancer cells in a mouse model. We also demonstrated that combination of MEK-inhibitors with neuraminidase-inhibitor oseltamivir have a synergistic effect against influenza virus *in vitro* and *in vivo*. We found that MEK-Inhibitors Cl-1040, RO5126766, PLX4032 and GSK-1120212 showed EC50 levels against influenza virus in the nM range and that combination increased the antiviral capacity of oseltamivir in A549 cells and in a mouse animal model. Most important, strong antiviral activity was also found when concentrations of MEK-inhibitors and oseltamivir were combined those were ineffective as single compound treatment. Our data gives rise to the assumption that antiviral drugs against influenza targeting cell signaling pathways might not in general toxic, because drug concentrations required for antiviral activity might be below the concentrations required for anti-cancer therapy.

74 Evaluating the Impact of Educational Interventions on Use of Highly Active Antiretroviral Therapy and Adherence Behavior in Indian Human Immunodeficiency Virus Positive Patients: Prospective Randomized Controlled Study

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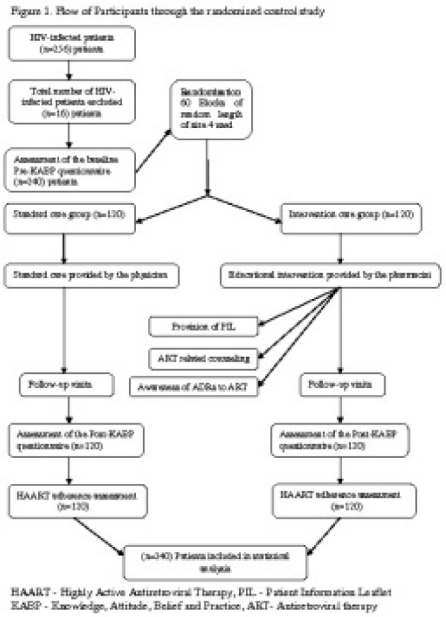
BACKGROUND: In India, poor adherence to antiretroviral therapy (ART) is due to lack of ART counseling leading to intentional non-adherence; thus, educational intervention (EI) must be sought. AIMS: The study was conducted to evaluate the impact of an EI on use of highly active antiretroviral therapy (HAART) and adherence behavior to HAART in Indian HIV-infected patients. MATERIALS AND METHODS: A prospective, randomized, controlled, interventional study to assess the impact of EI and counseling in-comparison to usual standard care was carried out from August 2009 to May 2012. The data were assessed for the baseline pre questionnaires scores for knowledge, attitude, belief and practice (KABP) responses. Patients under intervention care group (ICG) were offered with patient information leaflets (PIL), ART counseling on adherence and awareness of adverse drug reactions (ADRs) to ART by clinical pharmacist. Post-KABP responses was documented after follow-up visits in ICG and standard care group (SCG) and percentage of adherence from self-report were measured. ***Results:*** Among 240 patients, block randomization was used to assign the patients to the ICG and SCG. Analysis of variance test of pre and post KABP between ICG and SCG, Mean ± SD significantly increased ($p < 0.001$) in the ICG. Greater than 95% of HAART adherence; 81(67.5%, $p < 0.001$) was seen in ICG after EI compared to SCG 58(48.3%). CONCLUSION: Our study concludes EI by pharmacist was efficient for increasing HAART adherence, changed their negative beliefs, social stigma and intentional non-adherence behavior.



75 Distinct Inhibition of Nonstructural Gene Expression Potently Reduces the Propagation of Influenza A Virus *in vivo*

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BACKGROUND: Influenza A virus, a member of *Orthomyxoviridae* family, causes the most prevalent infection of the respiratory tract in humans. The viral genome mutates rapidly causing emergence of new strains against which there is no immunity in the existing population. This notorious nature of the virus calls for continuous development of new antiviral agents for effective prevention and management of influenza virus infection. In our study, we utilized the RNA interference approach for successful inhibition of influenza A virus replication in experimental mice. METHODOLOGY: We designed four sets of small interfering RNA (siRNA) against different regions of non structural gene conserved among various strains of influenza A virus. The siRNAs were administered in Balb/c mice using *in vivo* jet PEI reagent followed by virus infection. Mice lung homogenates were subjected to plaque assay, real time RT-PCR and immunoblot analysis for estimation of virus titer and gene expression. Thin sections of lungs were examined for histopathological changes by haematoxylin and eosin. Cytokine profiling was done in bronchoalveolar lavage samples by ELISA. Survival assay was done to assess the protective potential of the siRNAs. The specificity of siRNAs was confirmed by using mutated siRNA in each experiment. RESULTS: The expression of non structural gene of influenza A virus significantly reduced (upto 92%) in siRNA treated mice lungs. Potent decrease in lung virus titers and inflammatory damage was observed in the presence of siRNAs as compared to the

untreated control. The levels of TNF-α and IFN-γ cytokines reduced by 15-20 fold, whereas the levels of IFN-α1, IFN-β and IL-1β relatively increased in siRNA treated/ virus infected mice. The siRNAs conferred 100% protection to the virus infected animals. **Conclusion**: Our findings suggest that the siRNAs tested against nonstructural gene can significantly guard against influenza A virus infection in a dose-dependent pattern. These may be used as potential candidates for prevention and management of influenza virus infection.

76 Comparative *in vitro* Antiviral Activity of Multiple Classes of Influenza Inhibitors in Madin-Darby Canine Kidney Cells (MDCKs) and Normal Human Bronchial Epithelial Cells (NHBEs)

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Anti-influenza compound screening has been traditionally performed in MDCK cell line, but models based on primary respiratory epithelial cells would be a better representation of the physiological site of virus replication in humans. Here we report on the development of a novel anti-influenza screening assay in undifferentiated and fully differentiated NHBEs grown in an air-liquid interface culture system. Both influenza A and B strains efficiently replicate in NHBEs regardless of their differentiation status without causing significant cytopathic effects. Influenza replication in NHBE models can be reliably quantified by measuring the virus-associated neuramidase enzymatic activity or viral nucleoprotein levels in infected cultures. The antiviral activity of influenza inhibitors targeting viral M2 channel, neuraminidase, endonuclease, or RNA polymerase (2'-fluoro ribonucleosides and ribavarin) were similar in MDCKs as well as undifferentiated and differentiated NHBEs. While both the RNA polymerase inhibitor favipiravir (T-705) and its ribofuranose derivative (T-705-ribofuranose) showed potent antiviral activity in MDCKs (EC_{50} = 2.3 and 6.8 μM, respectively), only T-705-ribofuranose was active in NHBEs (EC_{50} = 17μM). Favipiravir showed no activity in NHBEs regardless of their differentiation status (EC_{50} >100μM). NHBEs treated with favipiravir accumulated the compound metabolites, including the active species 4-ribofuranosyl-5'-triphosphate (T-705 RTP), less efficiently than NHBEs treated with T-705-ribofuranose. In contrast, both favipiravir and T-705-ribofuranose were efficiently converted to T-705 RTP in MDCKs, HeLa cells, and primary murine bronchial epithelial cells. RNAi knockdown experiments in HeLa cells identified hypoxanthine phosphoribosyl transferase 1 (HPRT1) as an enzyme having a significant role in the initial intracellular metabolic activation of favipiravir. Interestingly, HPRT1 was found to be present both in MDCKs and NHBEs, suggesting that limited cellular uptake or excessive efflux of favipiravir rather than impaired metabolic activation may be responsible for its reduced antiviral potency in NHBE cultures.

77 Antiviral Assessment of Penultimate Methyl Alkyloxyalkyl Esters of Phosphonomethoxypropyl Nucleosides Against HIV-1 *in vitro*

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Esterification of CDV, HPMPA or PMPA with alkoxyalkyl groups leads to remarkable increases in antiviral activity. This is due primarily to increased cellular uptake and conversion to their diphosphates. Long hydrocarbon chains may be hydroxylated at the omega or omega-1 carbon atoms by omega oxidation. After further oxidations catalyzed by alcohol and aldehyde dehydrogenases, the omega carbon is converted to a carboxylic acid which undergoes cycles of beta oxidation leading to an inactive carboxylic acid metabolite. Hexadecyloxypropyl and octadecyloxyethyl groups were modified by introducing penultimate methyl groups. The omega-1 methyl alkanols were synthesized using Grignard reactions of isoamyl or isobutylmagnesium bromide and omega-bromo-alpha-alkanol. The alkanols were then converted to methansulfonates and treated with 1,3-propanediol or ethylene glycol to give a series of omega-1-methylalkoxyalkanols which were coupled to phosphonomethoxypropyl nucleosides (A, G and diaminopurine) using the Mitsunobu reaction (Ruiz, Bioorg Med Chem 19:2950,2011). Human PBMCs were infected with HIV-$1_{us/92/727}$ and treated with dilutions of drug. RT was measured in the culture supernatant and XTT was used to assess cell viability. HDP esters of PMPA, PMPDAP and PMPG were highly active with EC50s

ranging from 0.24 to 1.56 nM. The antiviral activity of ODE esters was similar (EC50s 0.23-2.83 nM). Penultimate methyl group did not generally cause any significant increase or decrease in antiviral activity. CC50 values for all compounds were >100 nM. In conclusion, introduction of penultimate methyl groups into the alkyl chain of alkoxyalkyl esters of PMP nucleosides does not have a deleterious effect on their antiviral activity against HIV-1. The antiviral activity of these analogs is substantial with EC50 values ranging from 0.23 to 7.3 nM. We have previously shown that penultimate methyl analogs of alkoxyalkyl acyclic nucleoside phosphonates slow human, monkey, rodent and guinea pig liver S9 metabolism of these agents versus their straight chain counterparts.

78 The Triethylbenzene Scaffold in the Search for Lectin Mimetics: A Novel Strategy for Anti-HIV Therapy

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The introduction of highly active antiretroviral therapy (HAART), in the late 1990s, has significantly contributed to reduce the morbidity and mortality due to infection with Human Inmunodeficiency virus (HIV), the retrovirus responsible of AIDS. However, long-term toxicities, drug-drug interactions, and the emergence and transmission of drug-resistant viral strains constitute a significant barrier to long-term successful treatment. Therefore, there exists an urgent need to discover new treatment options directed towards novel targets or towards currently validated targets but through mechanisms that are different from those employed by the clinically used anti-HIV drugs. In this sense, the initial steps in the HIV replicative cycle (entry and/or fusion with the host cells) are very attractive targets. Some lectins, carbohydrate-binding proteins of natural origin, show a potent inhibitory activity against HIV. The binding of these lectins with the glycans present in the glycoprotein gp120 of the viral envelope affects the interaction with the host cell surface preventing HIV transmission and entry into the host cells. Moreover, prolonged exposure to lectins results in the emergence of drug resistance. The mutations in the resistant viral strains predominantly affect *N*-glycosylation sites on gp120 and up to 16 different sites have been found deleted under lectin pressure. It has been hypothesized that such lectin-resistant strains might trigger a more effective immune response by the host. In the last years our research efforts have been focused on the discovery of synthetic small molecules, able to act against HIV through a mechanism similar to that of the natural lectins, therefore they could be ascribed as the so called "lectin mimetics". Inspired by the interactions established between lectins and glycosides, we have designed and synthesized a series of receptors with a central triethylbenzene core substituted with different aromatic entities rich in hydrogen-bond forming groups. The synthesis and anti-HIV activity of such compounds will be presented.

79 Inhibitory Potential of Azadirachta indica Juss (neem) Leaves on Influenza A Virus Replication

Latika Saxena, Roopali Rajput, Binod Kumar, Madhu Khanna
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Inhibitory Potential of *Azadirachta indica* Juss (neem) Leaves on Influenza A Virus Replication Latika Saxena*, Roopali Rajput, Binod Kumar, Madhu Khanna Department of Respiratory Virology, VP Chest Institute, University of Delhi, Delhi-110007

BACKGROUND: Influenza A viruses are known to cause widespread epidemics and pandemics with high mortality owing to their high rate of mutation. Rapid emergence of drug resistance against the currently available antiviral drugs and the limited use of influenza vaccines have exposed the need for the development of new and better antivirals against influenza. Many medicinal plants possess potent antiviral activities. In this study, we explored the antiviral activity of the leaf extract of *Azadirachta indica* (commonly known as "Neem"), against influenza A (H3N2) virus. MATERIALS & METHODS: Methanol extract of *Azadirachta indica* leaves was prepared and tested for its cytotoxic activity on Madin Darby Canine Kidney (MDCK) cells by colorimetric assay. In the dose response assay, different concentrations of the extract were tested for their inhibitory activity on A/Udorn/307/72

(H3N2) infected MDCK cells. The antiviral activity of the extract was further validated by the cytopathic effect (CPE), hemagglutination assay (HA), real time RT-PCR and western blotting. **Results:** The potent anti influenza effect of the extract was evident by the dose dependant reduction in CPE of MDCK cells and decrease in the HA titer. 50% cytotoxic concentration (CC_{50}) of the extract on MDCK was 60µg/ml and the median effective dose (ED50) was 50 µg/ml. There was 53% increase in the cell viability of the infected MDCK cells. Real time RT-PCR showed 40% inhibition in the expression of HA gene of the virus. The same was observed by western blot analysis. Time response study revealed that the antiviral activity of the extract was highest at 2-6 h post infection. **Conclusion:** The results of this preliminary study clearly reflect that the leaf extract of *Azadirachta indica* is able to inhibit the growth of influenza A virus. The present study conclusively reveals that *Azadirachta indica* leaves can be exploited in future for the development of an alternative and effective antiviral therapy against influenza A viruses.

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

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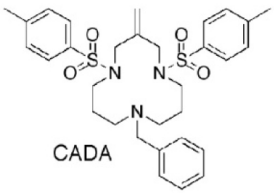
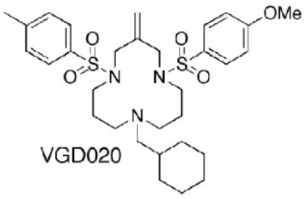
Cyclotriazadisulfonamide (CADA) inhibits HIV replication by selectively down-modulating expression of the CD4 receptor protein on host cells. Current studies are aimed at developing a prodrug approach involving various CADA analog prodrug parent compounds bearing dipeptide chains that are covalently bonded to two amino or two hydroxyl groups attached to the para position of the methylbenzenesulfonyl side arms. Cleavage of these chains by dipeptidyl-peptidase IV is expected to convert the prodrugs into various active CADA analogs. For this purpose, prodrug parent compound ES04 in which the methyl groups of CADA are replaced by aminomethyl groups has been synthesized. According to a 3D-QSAR model, ES04 is expected to have a CD4 down-modulation potency that is similar to that of CADA. Other prodrug parent compounds bearing N-hydroxyamino or O-aminohydroxy groups are current synthetic targets. The second objective of this work is to determine if the size of the macrocyclic ring is a contributing factor to potency for CD4 down-modulation. The CADA analog VGD020 possesses the greatest potency for CD4 down-modulation and HIV inhibition to date. The 12-membered ring size of VGD020 is being varied to 11-membered and 13-membered to produce novel analogs for testing.





81 Studies on HIV Integrase/Ledgf Inhibitory Activity of Compounds Isolated From Ethanolic Extract of Morinda Citrifolia L Noni

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BACKROUND: The development of antiviral drugs has provided crucial new means to mitigate or relieve the debilitating effects of many viral pathogens. A rich source for the discovery of new HIV infection inhibitors has been and continues to be, the 'mining' of the large diversity of compounds already available in nature and specifically those from botanical extracts. *Morinda citrifolia* is used in the Indian system of medicine for the treatment of variety of diseases including HIV/AIDS. Present work is to study HIV integrase and HIV Integrase/Lens Epithelium Derived Growth Factor (LEDGF) inhibitory activity of compounds isolated from ethanolic extract of *Morinda citrifolia* L Noni fruit. METHOD: Ethanolic extract of *Morinda citrifolia* fruit subjected to column chromatograpy for the isolation of the bioactive compounds (MCF ET C10-18) and isolated compounds have been studied against inhibition of HIV IN/LEDGF assay performed by Alpha Screen Technology and HIV-1 integrase enzymatic activity by oligonucleotide based assay, respectively.RESULTS: All the compounds except C-17, C-18

exhibited significant inhibitory activity against HIV Integrase/LEDGF (IC_{50}: 0.53-36 µg/ml). Compound MCF ET C-14 ME displayed potent inhibitory activity against HIV Integrase/LEDGF (IC_{50}:0.53 µg/ml) interaction. CONCLUSION: Anthroquinone, flavanoids and glycosides are the principle active constituents of ethanolic extract of *Morinda citrifolia*, which may responsible for HIV integrase/LEDGF inhibitory activity.

HIV Integrase and HIV IN/LEDGF Inhibitory Activity

Compounds	LEDGF IC_{50}[a] (µg/mL)	3'P IC_{50}[b] (µg/mL)	ST IC_{50}[c] (µg/mL)
MCF-ET-C-10-ME	14	>100	>100
MCF-ET-C-12-ME	10	>100	>100
MCF-ET-C-14-ME	0.53	94	58
MCF-ET-C-15-ME	36	>100	>100
MCF-ET-C-16-ME	31	>100	>100
MCF-ET-C-17-ME	>50	>100	>100
MCF-ET-C-18-ME	>50	>100	>100

The results are IC50 ±S.D, $n = 3$ for HIV-1 IN inhibitory activity [a]Concentration required to inhibits 3' processing reaction, [b]Concentration required to inhibits 3' processing reaction, [c]Concentration required to inhibits HIV IN/LEDGF interaction,

82 HIV RNase H Inhibitory Activity of Novel Heterocyclic Compounds

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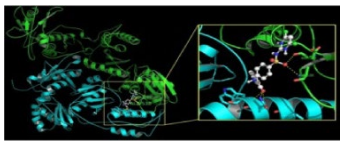
BACKROUND: Acquired immunodeficiency syndrome (AIDS) is a fatal pathogenic disease caused by Human Immunodeficiency Virus (HIV), a retrovirus. At this juncture designing of simple and novel molecules with broad-spectrum antiretroviral activity against HIV which is cheaper and affordable by the patients is essential. HIV RNase H is one of the attractive therapeutic targets for inhibition of virus infection, thus a potential inhibitor of HIV RNase H is provide additional candidate for control of HIV infection. Hence, the present is aimed at design and synthesis of novel heterocyclic compounds followed by screening for their inhibitory activity against HIV-RNase activity. METHOD: Series of novel heterocyclic compounds were synthesized and Investigated for inhibition of HIV-1 RNase H enzymatic activity and molecular modeling studies also performed by using computational methods to understanding the ligand-protein molecular interaction. RESULTS: Isatin derivatives (SPIII-5H) inhibits HIV -1 RNase H (8.4 µM) activity. From molecular modelling study indicates that the studied compounds bind with active site of HIV-1 RNase H. **Conclusion:** Isatin derivative (SPIII-5H) are the novel class of inhibitors of HIV 1 RNase H and this lead molecule is suitable for further molecular modifications.



Compound	HIV-1 RNase-H [a]IC_{50}
Q-PATH	> 100 (77%)[b]
Q-SDN	> 100 (100%)
Q-SMZ	> 100 (85%)
SPIII-SH	8.4
SPS-I	> 100 (100%)

83 Design, Molecular Modeling and Synthesis of Novel Isatine derivatives as Inhibitors of HIV Integrase/LEDGF Protein-protein Interaction

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BACKGROUND: HIV integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Previous studies have demonstrated that HIV-1 Integrase interacts with a cellular lens epithelium-derived growth factor (LEDGF/p75) and that this viral/cellular interaction plays an important role for tethering HIV-1 preintegration complexes (PICs) to transcriptionally active units of host chromatin. Small molecule inhibitors of HIV IN/LEDGF have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS. Present work is to Design, Synthesis, Molecular modelling and investigation of isatin derivatives as potential inhibitor of HIV Integrase/LEDGF interaction. METHOD: Novel isatin derivatives were synthesized and HIV Integrase/LEDGF interaction inhibition assay performed by using ALPHA screen technique. Hypothetical binding modes of the selected compounds in HIV integrase were generated using GLIDE docking tool. Results: Isatin derivatives (SP III-5H and SPIII-NA) inhibits HIV IN/LEDGF interaction and compound SPIII-5H more potent compound (15.1 μM). Molecular modeling studies indicate that the studied compounds can bind within the active site of HIV integrase (DDE) and thus interrupt the binding of HIV integrase with LEDGF. CONCLUSION: Isatin are the novel class of inhibitors of HIV IN/LEDGF interaction (protein-protein) and this lead molecule along with the residues identified through modeling stuides is suitable for further molecular modifications.

	HIV IN IC_{50} (μM)		LEDGF-IN
Compound	**3' Proc.**	**ST Proc.**	**α-screen (μM)**
SPIII-5H	9 ± 1	6 ± 1	15 ± 1
SPIII-NA	16 ± 5	9 ± 2	26 ± 4
SPIII-5Br	32 ± 5	11 ± 5	>100
SPIII-5Cl	67±11	46±11	>100
SPIII-5Me	>100	>100	>100
SPIII-5H-BZ	>100	95 ± 5	>100

84 Design and Synthesis of Quinazolin-4(3H)-ones as Novel inhibitors of HIV Integrase/LEDGF

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Background: During the early stage of HIV-1 replication, integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Previous studies have demonstrated that HIV-1 Integrase interacts with a cellular lens epithelium-derived growth factor (LEDGF/p75) and that this viral/cellular interaction plays an important role for tethering HIV-1 preintegration complexes (PICs) to transcriptionally active units of host chromatin. Small molecule inhibitors of HIV IN/LEDGF have emerged as promising new class of antiviral agents for the treatment of HIV/AIDS. Present work is to Design, Synthesis and investigation of Quinazolin-4(3H)-one derivatives as potential inhibitor of HIV Integrase/LEDGF interaction. Method: Quinazolin-4(3*H*)-one derivatives (SPS-I and II) were synthesized and HIV IN/LEDGF interaction inhibition assay performed by using ALPHA screen technique and molecular modeling studies also carried by using computational methods. Results: Quinazoline derivatives (SPS-I and II) inhibits HIV IN/LEDGF interaction and compound SPS-I more potent compound (9.23 μM). From molecular modelling study

indicates that the studied compounds bind with active site of HIV integrase (DDE), change the conformation and interrupt the binding of HIV integrase with LEDGF. Conclusion: Quinazolines are the novel class of inhibitors of HIV IN/LEDGF interaction (protein-protein) and this lead molecule is suitable for further molecular modifications.

Compounds	MW	Inhibitory (%) (@10μM)	LEDGF-IN (IC_{50} μM)
SPS-I	377.42	49.96	10.08 ±3.11
SPS-II	456.31	52.52	9.23±0.47
Compound 3**	313.74	44.07	11.91±0.61

HIV IN/LEDGF INHIBITORY ACTIVITY OF QUINAZOLIN-4(3*H*)-ONES
Quinoline derivative from Nat. Chem. Bio., 2010,6,442

85 Screening Small Molecule Inhibitors of Influenza-Host Protein Interactions

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The emerging resistance to current anti-influenza therapy has necessitated the identification of new targets and development of new therapies. Prosetta has established a unique moderate throughput screen (MTS) based on a cell-free protein synthesizing system that recreates viral nucleoprotein assembly pathways. The antiviral compounds identified target cellular factors, including those involved in Influenza A virus propagation. An earlier generation of the MTS achieved cell-free assembly of Influenza virus nucleoprotein (NP) into large complexes in an ATP-dependent fashion. More recently, based on improved understanding in the field of Influenza genome assembly, we incorporated Influenza matrix (M1) and polymerase (PA and PB2) subunits in addition to Influenza NP. This next-generation screen is hoped to more closely mimic the influenza virus nucleoprotein complex assembly in cells and may allow for a larger subset of cellular host factor engagement as targets for antiviral drug discovery. We will present the biochemical optimization of the cell free system comparing the first and second-generation screens. The hits from the primary screen of small molecule chemical library are currently being used in the pre-lead series screening in Influenza live virus cell culture assay. The small molecule hits from our first generation screen has allowed, through target identification, an important new observation: that the host multiprotein complexes are modified in infected versus uninfected cells. By analogy to a related screen for *Rhabdoviridae* we hypothesize that the targets include multiprotein complexes, and therefore that this approach identifies unconventional next generation targets.

86 Synthesis and Anti-HCV Activity of pyrazolo[3,4-d]pyrimidine Carbocyclic Nucleosides

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The Hepatitis C Virus (HCV) RNA-dependent RNA polymerase (RdRp) is an important target enzyme to discover RdRp inhibitor as direct acting antiviral (DAA). The promissing discovered molecules (I-III) belong to the prototype class of nucleosides. Recently, the carbocyclic nucleosides are considered as an interesting class of molecules to explore its potential as anti-HCV agent. The present studies were conducted via structure-based medicinal chemistry (SBMC) approach through the molecular modeling of enzyme as well as enzyme-inhibitor complex (RdRp, metal ions, short chain of template and primer, including incoming NTP), synthesis followed by anti-HCV evaluation. The conformation approaches were studied in detail for the comparision of pseudonucleoside with natural nucleoside, so that a suitable competetive inhibitor (**IV**) can be designed for synthesis. The designed carbocyclic nucleosides (**IV**) were docked into the active site of HCV RdRp and *in-silico* studies were conducted to select new analogs of pyrazolo[3,4-*d*]pyrimidine followed by synthesis. All the compounds were purified

and spectroscopically characterized for anti-HCV evaluation. The bromo analogs of pyrazolo[3,4-*d*]pyrimidine had shown EC_{50} of 6.6 µM and identified as potential lead for further optimization. Overall the present study utilizes RdRp-inhibitor complex as a model structure towards identifying a novel inhibitor. (Acknowledgement: Department of Science and Technology, Government of India for financial support: SR/FT/CS-001/2009)

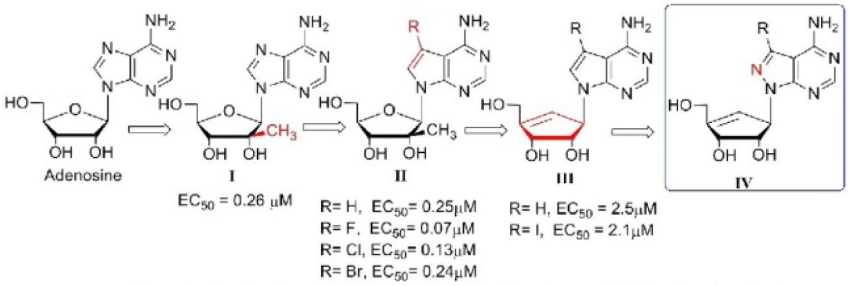

Figure 1: A rational structural modifiction for creation of new anti-HCV lead analogs IV.

87 Antiviral Activity of Usnic Acid Derivates Against Influenza A/Aichi/2/68 *in vivo*

Anna A. Shtro[1], Olga A. Luzina[2], Marina P. Polovinka[2], Dmitry N. Sokolov[2], Nina I. Komarova[2], Nariman F. Salakhutdinov[2], Oleg I. Kiselev[1], Vladimir v. Zarubaev[1]

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BACKGROUND. Despite success in chemotherapy and vaccine prophylaxis, influenza remains a poorly controlled infection causing annual epidemics and pandemics. Usnic acid (UA), a dibenzofuran originally isolated from lichens has been shown previously to act as a growth regulator in higher plants. In humans, it can possess anti-inflammatory, antimitotic, antineoplasic, antibacterial, and antimycotic activities. The aim of this study was to evaluate anti-influenza properties of some derivatives of usnic acid.

MATERIALS AND METHODS. We had tested four compounds: (+) and (-) isomers of usnic acid and (+) and (-) isomers of its derivative (labelled as 575 and 612, respectively) on a model of lethal influenza pneumonia in mice (influenza virus A/Aichi/2/68 (H3N2). Compounds 575 and 612 were chosen after previous testing of the antiviral activity of 30 usnic acid derivates *in vitro*. All the substances were synthesized in Novosibirsk Institute of Organic Chemistry. The drugs were administered in two doses, 0,5 and 0,25 LD50 (50 % lethal doses). The virus A/Aichi/2/68 (H3N2) was administered intranasally at 1 and 10 LD50 under slight ether anesthesia. The following treatment scheme was used: 0.2 mL of a drug water solution was administered one day before infection, 1-2 hours after infection and daily afterwards for 4 days. The animals were observed for 14 days, and deaths in the control and experimental groups were reported every day. Based on these data, the degree of animal protection was calculated.

RESULTS. Both forms of usnic acid had shown no protection. Compound 612 had 11,1% of protection in both doses, but the best one was 575. The maximum level of protection of 33,3% was achieved when the animals were receiving 10 mg/kg of the substance daily.

CONCLUSIONS. Thus, compounds 612 and 575 can be considered as a prospective ones for prevention and/or treatment of influenza.

88 Evaluation of Acrylamide Grafted Sago Starch for Controlled Delivery of Anti-HIV Drug

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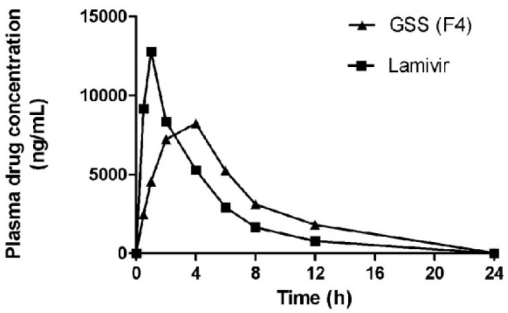
In the present investigation an attempt has been made to develop a new controlled release polymeric material and it was also evaluated were for preparing controlled release tablets of lamivudine using this novel polymer. The acrylamide grafting was successfully performed on the backbone of sago starch. The modified starch was tested for acute toxicity and drug-excipient compatibility study. The formulation were evaluated for physical characteristics like hardness, friability, % drug content and weight variations. The *in vitro* release study showed that the optimized formulation exhibited highest correlation (R) value in case of higuchi model and the release mechanism study proved that the formulation showed a combination of diffusion and erosion process.There was a significant difference in the pharmacokinetic parameters (T_{max}, C_{max}, AUC, V_d, $T_{1/2}$ and MDT) of the optimized formulation as compared to the marketed conventional tablet Lamivir®.



89 Small Molecule Allosteric Activation of APOBEC3G as a Prophylactic Against HIV Infection

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APOBEC3G (A3G) is a mutagenic cytidine deaminase enzyme that converts dC to dU in single stranded DNA during HIV replication and thereby serves as a host-defense factor against HIV infection. The current hypothesis in the field is that A3G exerts its antiviral activity by incorporating with virions and mutating the HIV genomic sequence during reverse transcription. However HIV encodes its own protection in the protein known as Vif that degrades A3G during late infection before it can assemble with viral particles. It has been suggested that pre-existing cellular A3G also may be able to preemptively strike down incoming virus were it not for RNA-dependent inactivation of A3G as ribonucleoprotein particles (RNP) in the target cell cytoplasm (Smith (2011), TiBS 36:239-44). We show here that A3G forms distinctly different complexes with RNA than it does with ssDNA and that cellular RNAs allosterically inhibit the ability of A3G to both bind to ssDNA substrate and catalyze dC to dU deamination. Using molecular FRET in high throughput screening, small molecules have been identified that selectively reduced RNA binding to A3G and activated A3G ssDNA deaminase activity. Cells were tolerant of these compounds to the uM level however viral infectivity was markedly inhibited in cells pretreated with nM concentration of these compounds. Liberating A3G from cellular RNP also enhanced A3G assembly with virions; thereby inhibiting the spread of an infection. These data show for the first time that small molecule A3G activators (SMAA) can be used to inhibit infection of incoming virus based on A3G expressed in target cells. These findings suggest that oral or topical administration of SMAA may be an effective strategy for HIV therapy and prophylaxis, and with Vif antagonists suggest a possible path to a cure.

90 Adding of Interferon-gamma to Interferon-alpha and Ribavirin Increase the Efficacy of Chronic Hepatitis C Patients Therapy

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Despite the antiviral therapy of chronic hepatitis C (HCV) including interferon (IFN) – alpha and ribavirin has been considered to be a standard, many patients still remain resistant. The efficacy of IFN-gamma in some infectious and non-infectious diseases treatment has been shown recently. The task of this study was to evaluate the efficacy and safety of IFN-gamma (Ingaron), IFN-alpha and ribavirin in combination therapy of HCV patients.

There were 150 HCV patients aged from 18 to 50 years under our observation with moderate or high viral load, at 75% of patients HCV genotype 1 was found. All the patients were randomized for three groups (50 patients in each). First group patients were administered IFN-gamma 500 000 IU and IFN-alpha2b 3 mln. IU i/m three times a week, as well as second group received the same daily therapy. Third group patients were administered IFN-gamma 500 000 IU and IFN-alpha2b 3 mln. IU i/m three times a week with daily body weight ribavirin during three months. Clinical improvement was evident in all patients: normalization of ALT, serum bilirubin, cholesterol and alkaline phosphatase levels were shown. 34% of the first group patients, 26% of the second group patients and 96% of the third group patients had developed end-treatment virological response. No severe side effects were apparent in all patients. These data suggest that including IFN-gamma in the standard scheme of HCV therapy by IFN-alpha and ribavirin significantly increased end-treatment virological response and well-tolerated. So, IFN-gamma seems perspective for use in three-component combination therapy of HCV patients resistant to standard schemes including IFN-alpha and ribavirin.

91 A Cross-sectional Analysis of Antiviral in the First Cohort of HIV-Infected Children in North Eastern Uganda

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The impact of extensive use of ART in developing countries has been enormous. A thorough understanding of all factors contributing to the success of antiretroviral therapy is required. This study aimed at investigating the value of cross-sectional drug resistance monitoring using DNA and RNA oligonucleotide ligation essays (OLA) in treatment cohorts in low-resource settings like in Uganda. The study was conducted in the first cohort of children gaining access to structured ART in the Northern Region of Uganda. Methods 46 eligible children started the standard regimen of AZT, 3TC and NFV between 2008– 2011, Patients had a median age of 5.6 years (range: 0.7-14y), a median viral load of 1.7 ^{5} RNA/ml (range: 2.1 ^{3} – 1.2 ^{6}), and a median CD4-count of 232 cells/µL (range: 1–1591). Of these, 20 patients were classified as CDC clinical category C and 31/46 as CDC immune category 3. At the time of cross-sectional analysis in 2011, adherence questionnaires were administered. DNA OLAs and RNA OLAs were performed from frozen PBMC and plasma, RNA genotyping from dried blood spots. Results During the first year of ART, 44% of children experienced virologic failure, with an additional 9% failing by the end of the second year. Virologic failure was significantly associated with the number of resistance mutations detected by DNA-OLA ($p < 0.001$) during cross-sectional analysis, but also with low immunologic CDC-scores at baseline ($p < 0.001$). Children who had been exposed to unsupervised short-term antiretroviral before starting structured ART showed significantly higher numbers of resistance mutations by DNA-OLA ($p = 0.01$). Detection of M184V (3TC resistance) by RNA-OLA and DNA-OLA demonstrated a sensitivity of 0.93 and 0.86 and specificity of 0.67 and 0.7, respectively, for the identification of virologic failure. The RT mutations N88D and L90M (NFV resistance) detected by DNA-OLA correlated with virologic failure, whereas mutations at RT position 215 (AZT resistance) were not associated with virologic failure.

CONCLUSIONS: Advanced immune suppression at baseline and previous exposures to unsupervised brief cycles of ART significantly impaired treatment outcomes at a time when structured ART was finally introduced in his cohort. Brief maternal exposures to with AZT +/– NVP for the prevention of mother-to-child transmission did not affect treatment outcomes in this group of children. DNA-OLA from frozen PBMC provided a highly specific tool to detect archived drug resistance. RNA consensus genotyping from dried blood spots and RNA-OLA from plasma consistently detected drug resistance mutations, but merely in association with virologic failure.

92 Mutational Analysis of the Binding Pocket of Diketoacid L-742,001 in the Influenza PA Endonuclease

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The influenza virus PA endonuclease, which cleaves capped host pre-mRNAs to initiate synthesis of viral mRNA, is a prime antiviral target. This enzymatic reaction is strongly inhibited by the diketoacid L-742,001 (Merck). There is little information on its precise binding mode or resistance profile, i.e. which resistance mutations in PA may be involved and their impact on viral fitness.

We performed computer-assisted docking of L-742,001 into published PA crystal structures, combined with a comprehensive mutational analysis, to identify the residues within the PA active site involved in binding of L-742,001. Whether using a rigid or flexible protein docking approach, a similar binding mode of L-742,001 was predicted with a role for several hydrophobic residues surrounding the catalytic site.

Next, we created a series of mutant PA expression plasmids encoding a larger hydrophobic, hydrophilic or charged residue at the predicted L-742,001 binding sites. The effect of these mutations on the antiviral activity of L-742,001 was studied in two cell culture assays. Mutant influenza viruses, obtained by reverse genetics, were evaluated in a virus yield assay. Second, the luciferase-based viral ribonucleoprotein (vRNP) reconstitution assay was used to assess the impact of the mutations at the level of the vRNP complex.
Several residues in PA were proven to play a key role in its interaction with L-742,001 since mutating these sites resulted in a decrease (>20-fold) or, inversely, an increase in the antiviral activity of L-742,001. Consistent with the conserved nature of some of the predicted binding residues, mutations at these sites caused a drastic reduction in the functionality of the influenza polymerase and/or viral replication fitness.

Our studies provide unique insight into the precise binding mode of L-742,001 in the PA endonuclease active site. In addition, they establish the basis to explain the structure-activity relationship of diketoacid inhibitors of PA and guide further design of novel derivatives with improved binding to the PA active site.

93 Effects of Oseltamivir Treatment on Cytokine Production During an Influenza A/CA/04/2009 (H1N1) Virus Infection in Mice

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The dose responsive effects of oseltamivir treatment on cytokine production were evaluated during an influenza A/California/04/2009 (pandemic H1N1) virus infection in BALB/c mice. Mice were treated with 0.1, 1.0, and 10.0 mg/kg/day oseltamivir *per os* twice daily for 5 days starting 4 hours post-virus challenge. Survival following treatment was dose responsive with mean day of death values of 7.7, 8.8, 18.4, and 18.7, for placebo, 0.1, 1.0, and 10 mg/kg/d treatments, respectively. Virus titers and cytokine measurements were completed on lung lavage samples at days 1, 2, 4, and 6 post-infection. Virus titers peaked 1 day post-infection for the placebo and 10 mg treatment groups at 7.5 and 6.8 $\log_{10}$/ml. Where as, virus titers peaked at 7.2 $\log_{10}$/ml on day 4 for the 1 mg treatment group, and titers reached 7.2 $\log_{10}$/ml on days 2 and 4 for the 0.1 mg treatment group. Cytokine measurements revealed a significant increase, compared to placebo, in IL-1α and IL-1β for the 1 mg treatment group, and in IL-1α for the 0.1 mg treatment group on day 1. On day 2 post-infection, treatment with 10 mg oseltamivir led to significant decreases in IL-1α, IL-1β, IL-2, IL-10, IFN-γ, TNFα, MCP-1, MIP-1α, and RANTES. Treatment with 1.0 mg oseltamivir gave a mixture of increased (IL-4, IL-5, and IL-17) and decreased (IL-1α, IL-2, IL-10, and IFN-γ) levels of cytokines on day 2, but the 0.1 mg treatment mirrored the 10 mg dose in all cytokine levels except IL-1α and RANTES. In addition, a significant increase in IL-6, IL-10, and IFNg was observed for the 0.1 mg treatment group on day 2. On day 4 post-infection, significant increases in IL-10 were observed in the 0.1 and 1.0 mg treatment groups and increased levels of IFN-γ were observed for all groups. On day 6 post-infection, IL-2, IL-10, and RANTES increased, while IL-17 and IFN-γ decreased in the 1.0 mg treatment group. Treatment with 0.1 mg produced a decrease in IFN-γ and an increase in RANTES on day 6. These data show that the greatest number of significant changes in cytokine levels occurs on day 2 post-infection, and that changes in cytokine levels are related to virus titers. [Supported by Contract N01-AI-30063, Virology Branch, DMID, NIAID, NIH]

94 Inhibition of Hepatitis B Virus Replication by Analogs of HIV RNAseH and Integrase Antagonists

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Nucleos(t)ide analog drugs can block DNA synthesis by the Hepatitis B Virus (HBV) reverse transcriptase and can control the infection indefinitely. However, treatment rarely cures the infection, so therapy is life-long, has high costs and may have unpredictable long-term side effects. The profound suppression of HBV by the nucleos(t) ide analogs and their ability to cure some patients indicates that they can push HBV to the brink of extinction. Consequently, more patients could be cured by suppressing HBV replication further using a new drug in combination with the nucleos(t)ide analogs. The HBV ribonuclease H (RNAseH) is a logical drug target because it is the second of only two viral enzymes that are essential for viral replication, but it has not been exploited because it is very difficult to produce active enzyme for drug screening. We recently expressed active recombinant HBV genotype D and H RNAseHs and characterized the enzymes in preparation for drug screening. A small set of candidate HBV RNAseH inhibitors was identified using chemical structure-activity analyses based on inhibitors of the HIV RNAseH and integrase enzymes because RNAseH and integrase enzymes are members of the nucleotidly transferase superfamily and hence share similar protein folds and enzymatic mechanisms. Over 50% of the compounds inhibited the HBV RNAseH at 10 μM, the best compounds had low micromolar IC_{50} values against the RNAseH in biochemical assays, and about 10% of the compounds inhibited HBV replication in tissue culture at 10 μM. Inhibition of viral replication was confirmed to be via inhibition of the viral RNAseH activity. This study demonstrates for the first time that: 1) Recombinant HBV RNAseH suitable for low-throughput antiviral drug screening can be produced; 2) HBV replication can be pharmacologically inhibited by targeting the RNAseH; and 3) Inhibitors of the RNAseH can block HBV replication of multiple viral genotypes. The high very percentage of compounds developed against the HIV RNAseH and integrase that were active against the HBV RNAseH indicates that the drug development efforts against these HIV enzymes could guide and accelerate anti-HBV RNAseH drug discovery.

95 Development of a Robust RSV Replicon Assay for High-Throughput Screening

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Respiratory Syncytial Virus (RSV) is a major cause of lower respiratory tract infections in people of all ages especially in young children, elderly and immunocompromised patients. Identification of effective RSV inhibitors is urgently needed but has been hindered by the lack of a robust assay that is feasible for high-throughput screening (HTS) of large compound libraries. Here, we present the development and optimization of a 384-well RSV replicon assay that enabled HTS for RSV replication inhibitors with minimal biocontainment. This assay took advantage of a previously established stable RSV replicon cell line containing a luciferase reporter gene. Through the use of cryopreserved and enriched replicon cells, assay-ready compound plates, and optimized assay conditions, the screen was successfully automated with significant improvement in assay signal, reproducibility and throughput. The optimized RSV replicon assay demonstrated low signal variation, with calculated Z' » 0.6 across 200 assay plates and a signal to background ratio of >40. A validation screen was performed with 7,000 compounds in duplicate at 10mM and demonstrated high assay reproducibility. The dose response replicon assay was further validated with different classes of RSV replication inhibitors, and our data showed the replicon EC50s are comparable to EC50 values obtained from other RSV infection assays. This fully optimized RSV replicon assay has enabled multiple HTS campaigns for RSV replication inhibitors and our preliminary screen results showed the assay is robust and very reproducible with high hit confirmation rates.

96 2'-Fluoro-2'-deoxypurineriboside ProTides: A Step Forward Towards Developing Influenza Virus Polymerase Inhibitors

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The potential use of nucleoside analogues for the treatment of influenza virus infections is hardly explored. We here determined the inhibitory effect of a series of base- or ribose-modified GTP derivatives on influenza virus RNA polymerase, using an RNA elongation assay with virion-derived viral ribonucleoproteins (vRNPs). The most potent inhibitors of [8-^{3}H]-GTP incorporation were 7-deaza-GTP and 2'-fluoro-2'-deoxy-GTP, with IC_{50} values of 4.1 and 3.7 μM, respectively, compared to 37 μM for the obligate chain terminator 3'-deoxy-GTP. 7-Deaza-GTP proved to be an efficient alternative substrate, and supported further RNA elongation after its incorporation. In contrast, 2'-fluoro-2'-deoxy-GTP acted as chain terminator. This explains the anti-influenza virus activity of 2'-fluoro-2'-deoxyguanosine (FdG) *in vitro* and *in vivo*, as reported by others. To improve its intracellular disposition and activation, we applied the double prodrug approach, combining a ProTide motif for direct delivery of the nucleoside 5'-monophosphate and a 6-*O*-modified guanine to increase lipophilicity and cellular uptake. Several 6-*O*-Me, 6-*O*-Et or 6-Cl-modified FdG ProTides displayed activity in a PCR-based virus yield assay, causing a 2-$\log_{10}$ reduction in virus titer at ~12 μM. Similar data were obtained in the vRNP reconstitution assay, a cell-based viral RNA polymerase inhibition test. The parent nucleoside analogues had no anti-influenza virus activity at 100-200 μM. Metabolism experiments with carboxypeptidase Y or whole cell lysates showed that the FdG ProTides are readily cleaved to release the 6-*O*-modified FdG-5'-monophosphate. Efficient removal of the 6-*O*-substituent on the guanine part was suggested by enzymatic studies with adenosine deaminase, and by molecular modelling of the nucleoside 5'-monophosphates in the catalytic site of a model of ADAL1. Our results set the stage for developing novel ProTide inhibitors of influenza virus, using any of the successful substitutions examined here (i.e. 2'-fluoro 7-deazaguanine or a 6-*O*-modified guanine) as a relevant starting point. [Reference: Meneghesso *et al.*, ChemMedChem., 2013]

97 Modification of CCL5/RANTES Hot Spots Delivers Potent Anti-HIV-1 Full-length and Peptide Derivatives Acting as CCR5 Antagonists

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CCR5, the major HIV-1 co-receptor, is a primary target for HIV-1 entry inhibition strategies. Antagonism towards CCR5 is an important requisite, as receptor activation could lead to pro-inflammatory conditions that might even favor enhancement of HIV-1 transmission. Moreover, CCR5 antagonists are likely to promote anti-inflammatory effects, widening their therapeutic window. CCL5/RANTES, a natural CCR5 ligand, is one of the most potent HIV-1 entry inhibitors and therefore an ideal candidate to derive HIV-1 blockers [1]. Through a rationally-designed molecular evolution of RANTES, anti-HIV-1/CCR5-interacting hot spots within the original chemokine sequence have been extensively modified to increase anti-HIV-1 activity and convert a CCR5 agonist into an antagonist. RANTES engineering has been pursued on two molecular fronts: full-length mutants and peptide derivatives. R4.0, our most potent peptide derivative, presents cross-clade HIV-1 blocking activity (a feature shared with the peptide-based drug T20) and acts as CCR5 antagonist (similarly to the anti-HIV-1 drug Maraviroc) [2]. CCR5 antagonist full-length RANTES mutants have been produced that include a mosaic of hot spot mutations leading to an extremely potent anti-HIV-1 derivative (IC50% ~ 5 pM in HIV-1 BaL acute infection assays). Production and selection of RANTES variants through the engineering of commensal lactobacilli was ideal for the live microbicide concept [3] and as a recombinant protein screening platform [4]. Alternative production systems are being explored to attain higher amounts of these RANTES mutants in order to investigate them in different therapeutic contexts. Both (full-length and peptide) RANTES derivatives presented additivity/synergy effects when tested in combination with an array of different HIV-1 blockers, thus expanding their potency and lessening the risk of drug resistance. [1] Vangelista *et al.* Vaccine 2008 26:3008-15. [2] Secchi *et al.* Chem Biol 2012 19:1579-88. [3] Vangelista *et al.* Antimicrob Agents Chemother 2010 54:2994-3001. [4] Secchi *et al.* Protein Expr Purif 2009 68:34-41.

98 Optimizing the Unsymmetrical Structure of Benzyl-tailed CADA Analogs To Improve Their CD4 Down-modulating and Anti-HIV Activity

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CD4 is a crucial component of the cellular receptor complex for Human Immunodeficiency Virus (HIV). Thus, regulating CD4 expression can be an important target for antiviral intervention. We have previously reported that cyclotriazadisulfonamide (CADA) down-modulates CD4 expression in immune cells and by this means blocks HIV entry. Structural modifications of CADA have been made to increase potency, reduce toxicity, and improve physical properties. In contrast to the symmetrical structure of the lead compound CADA, a new set of CADA analogs was designed to contain modifications in one sulfonamide side arm while keeping the other side arm fixed as p-toluenesulfonamide. Seventeen new unsymmetrical CADA compounds, bearing a benzyl tail group, were synthesized to determine if side-arms other than 4-methoxysulfonamide lead to higher potency and to explore the effect of the electronic density of the second arenesulfonyl side-arm on activity. The compounds were tested for their CD4 down-modulating potency in CD4-transfected CHO cells by flow cytometry. In accordance with our previous studies, the open chain precursors lacked any CD4 down-modulating activity, confirming that the presence of an intact macrocyclic ring is crucial for potency. Removal of one of the p-toluenesulfonamide side arms was detrimental for activity, suggesting that both side-arms of the drug are needed for interaction with its (yet unknown) target protein. Of the other 16 unsymmetrical CADA compounds, 14 exerted CD4 down-modulating activity (IC_{50} ranged between 0.14 and 7.3 μM). Interestingly, the two analogs that showed a higher activity as compared to the lead compound CADA, were bearing a side-arm with high electron density. In addition, the CADA analogs were evaluated for their anti-HIV activity in TZM-bl cells. A correlation between the CD4 down-modulating capability and anti-HIV activity was observed. In conclusion, our data on the unsymmetrical CADA analogs suggest a bimolecular binding model for CADA and show that decreased symmetry may likely lead to improved activity of the compounds. This opens the road for new structures of unsymmetrical CADA analogs that may be explored.

99 New Approach to Personalized Mono- and Combination Therapy of Chronic Hepatitis C Patients

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Decrease of value for plasma/serum protein and non-protein thiols or blood thiol-disulfide (SH/SS) ratio is considered as biomarker of impaired immune function playing important role in pathogenesis of viral infections (Pero R., 1996 Sokolovsky V., 1996). The aim of this investigation was to study influence of interferon (IFN)-alpha, PEG-IFN, IFN-gamma and ribavirin and their combinations *in vitro* on the blood SH/SS ratio of patients with chronic hepatitis C (HCV) and to compare its *in vitro* and *in vivo* efficacy. Method for Screening Drug Preparations (US 6,627,452 EP 1,182,455 RU 2,150,700) based on testing of drug's *in vitro* influence on blood SH/SS ratio measured by spectrophotometry was employed. There were 115 patients with HCV in age of 16 – 60 years under observation. It was revealed that HCV patients with genotype 1b were *in vitro* significantly less sensitive to one of IFN-alpha2b preparation (16.7%) than patients with other (1a, 2 and 3) genotypes (70.6%). Each patient of investigated group was tested for sensitivity to two different IFN-alpha preparations and doses with choice of optimal drug and dose and its correction during treatment received personalized mono-therapy by IFN-alpha in doses 0.5/1.0/2.0/3.0 MU (60 patients). Second (control) group received standard therapy by recombinant interferon-alpha2b in dose 3MU 3 times per week (25 patients). The rate of complete response after 6-month personalized IFN-alpha mono-therapy was 82.8%, SVR after 6-month and 12-month follow-up – 75.9% and 62% correspondingly. The rate of side effects was 12% (in comparison with 75% in corresponding control group, $p< 0.01$). The same method was used for personalized combination therapy of 30 HCV patients by IFN-alpha + ribavirin, PEG-IFN + ribavirin, IFN-alpha + IFN-gamma, IFN-alpha/PEG-IFN + IFN-gamma + ribavirin. There were noticed correlations between *in vitro* and *in vivo* efficacy, in the cases of resistance to standard combinations IFN-alpha/PEG-IFN + ribavirin adding IFN-gamma was effective. Thus, proposed method can be used for *in vitro* diagnosis of resistance/sensitivity HCV patients to antiviral drugs and personalized therapy by IFN-alpha or PEG-IFN alone or in combinations with ribavirin and/or IFN-gamma.

100 Thiol-disulfide Ratio as Universal Biomarker for Screening Antiviral Drugs for Personalized Therapy

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The purpose of this study was the search of new biomarkers for personalized therapy of viral infection. Currently, a value for total plasma/serum thiols including both protein and non-protein thiols and blood thiol-disulfide (SH/SS) ratio is considered as proofs of the immune competency and a state of an organism nonspecific resistance. We have proposed an original method for screening drugs *in vitro* for their effect on the SH/SS blood ratio (US 6,627,452; RU 2,150,700). After 1 h incubation with drugs, the blood of test and control tubes are hemolyzed by lysator, and amounts of SH- and SS-groups are determined by spectrophotometry using Ellman's method and SH/SS ratio is calculated. There were 115 patients with chronic HCV-infection and 175 patients with genital HSV-infection under observation. Increase SH/SS ratio after drugs adding correlated with interferon-inducing activity of blood leukocytes, stimulation of mononuclear antiviral resistance and functional activity of monocytes and can be considered as biomarker of drugs' effectiveness. Decrease SH/SS ratio after antiviral drugs adding supported cytotoxic drug effect and can be considered as biomarker of drugs' toxicity. There was an excellent correlation between *in vitro* and clinical results. Two controlled clinical trials at HCV in adults shown 3-fold higher efficacy the of the interferon-alpha (IFN) mono-therapy used by personalized way in comparison with standard therapy and 6-fold decrease of side effects (12% vs.75%) . Remission rate was 75.9% vs.6% after 6-month and 62% after 12-month follow-up. Controlled study of the effectiveness of a personalized selection of antiviral and immune preparations at patients with genital HSV-infection showed an increase in the effectiveness of treatment of 15.9%. Thus, SH/SS ratio can be used as universal biomarker for screening antiviral drugs for personalized mono- and combination therapy to develop an original design of clinical trials. The method has also proven to be an excellent tool in a search for new antiviral therapies.

101 *In Vitro* Anti-Influenza Virus Activity of Extracts of *Sida cordifolia* L.

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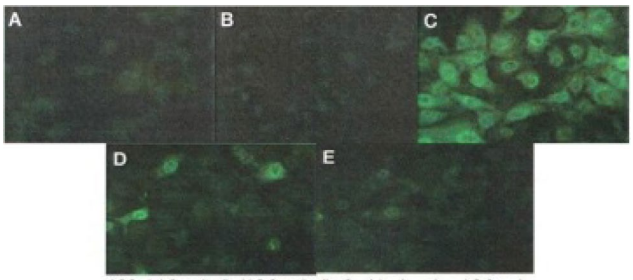
The H1N1 influenza virus has recently spread worldwide. The drugs used for the treatment leads to viruses resistant thus, it is important to develop new types of anti-influenza virus agents with action different from those of the known agents. In the present investigation the extracts of the whole plant of *Sida cordifolia* l. were examined for potential antiviral effect against the wild type of oseltamivir- resistant A/PR/8/34 (H1N1) and pandemic IFV-296 (H1N1) in plaque reduction or yield reduction assay. The mode of anti-influenza virus action was assessed by a virus adsorption assay, immunofluorescence assay of viral antigens and percentage virus inhibition was assessed by MTT antiviral assay. The results were expressed as 50 % cytotoxicity (CTC_{50}) for MTT assay and 50 % effective (EC_{50}) concentration for plaque reduction assay, and the selectivity index ($SI= CC_{50}/EC_{50}$) was calculated. From the six extracts tested hydroalcoholic (HA) and toluene (T) extracts showed highest SI, 41 and 35 against IFV-296 H1N1 virus respectively. In direct immunofluoroscence assay the viral antigens were strongly detected in the absence of HA and T extracts, as compared with the mock-infected cells and infected cells immediately after adsorption however, some viral antigen were detected in presence of plant extract at 50 µg/ml and they were negligible at 100 µg/ml. HA and T extracts was suggested to suppress the expression of viral antigen in the infected cells (Figure 1). **Conclusion:** HA and T extracts suggested to have anti-influenza activity *in vitro*. Further in depth *in vivo* anti-influenza virus studies are progressing in our laboratory.



a) Mock infected cells. b) Infected cells after 1 h adsorption. c) Infected cells in the absence of plant extract. d) HA at 50 mcg/ml. e) HA at 100 mcg/ml

102 Synthesis of Novel 2-Cyano7-Deaza-8-Azapurine Derived Nucleosides and Their Activity Against HCV

Philip Wainwright[1], Adrian Maddaford[1], Mike Simms[1], Xiurong Zhang[1], Neil Forrest[1], David C. Pryde[2], Scott C. Sutton[3], Mark S. Betson[1]

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As part of a program to generate a 1000 compound library of nucleosides intended for screening against HIV and HCV, we were interested in synthesising 2-cyano-7-deaza-8-azapurines as potential adenine mimics. This poster will describe a novel and robust synthesis of 6-chloro-2-cyano-7-deaza-8-azapurine, utilising a PMB protecting group at the N9 position to allow for the incorporation of the nitrile group in an efficient high yielding manner. It will then be demonstrated how this adenine mimic was coupled to an array of sugars at the N9 position, and amino substituents introduced at the C6 position, with particular consideration being paid to the reactive nitrile functional group. Finally, and for the first time, data will be presented for the activity of these novel nucleosides in a cell based HCV replicon assay. Reference: Wainwright, P., Maddaford, A., Simms, M., Zhang, X., Leese, D., Glen, R., Hart, J., Forrest, N., Pryde, D.C., Middleton, D.S., Stephenson, P.T., Guyot, T., Sutton, S.C. *Synlett*, **2011**, 1900.

103 Novel 2,4-diaminopyrimidine Nucleoside Phosphonate Antiviral Prodrugs

Melissa M. Williams[1], Boris A. Kashemirov[1], Marcela Krecmerova[2], Tomas Tichy[2], Mark N. Prichard[3], John C. Drach[4], John M. Hilfinger[5], Charles E. McKenna[1]

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The DAPy class of acyclic nucleoside phosphonate (ANP) analogues, where the base is a 2,4-diaminopyrimidine (DAPy), and the aliphatic phosphonate side chain is connected via an ether linkage at the C-6 position of the pyrimidine ring, was first announced by the Holý laboratory in 2002. (*R*)-HPMPO-DAPy, PMEO-DAPy and (*R*)-PMPO-DAPy have been found to exhibit an antiviral activity spectrum similar to their purine (*S*)-HPMP-, PME- and (*R*)-PMP-ANP counterparts. While DAPy parent drugs have demonstrated antiviral activity worthy of further exploration, they are limited by poor permeability due to the presence of the free phosphonic acid. We previously reported the synthesis of the prodrugs of (*S*)-HPMPA and (*S*)-HPMPC (cidofovir, Vistide®), by conjugating a non-toxic amino acid or dipeptide to the phosphoryl group as an ester linkage with a serine, threonine or tyrosine side chain hydroxyl group. Several of these tyrosine-based derivatives exhibit good stability, significantly enhanced antiviral activity and increased oral bioavailability compared to the parent drugs. We present here the synthesis and a preliminary *in vitro* antiviral evaluation of a small collection of tyrosinamide-based prodrug esters of PMEO-DAPy and (*R*)-HPMPO-DAPy against HSV-1, HCMV and VV. Prodrugs USC-544, USC-545 and USC-556 demonstrate EC_{50} values 17-100 fold greater than the parent drug.

ACKNOWLEDGEMENTS: This work was supported by NIH grant R43AI091216, NIH grant R43AI100401, NIH contract HHSN272201100016I, AMVIS grant ME10040 (Ministry of Education, Youth and Sport of the Czech Republic) and Academy of Sciences of the Czech Republic grant M200551201.

104 The Griffithsin Dimer is Required for High Potency Inhibition of HIV-1

Jie Xue[1], Bart Hoorelbeke[2], Ioannis Kagiampakis[1], Borries Demeler[3], Jan Balzarini[2], Patricia J LiWang[1]
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Griffithsin (Grft), a protein lectin derived from red algae is among the most promising antiviral proteins yet discovered. It has broad activity against a variety of viruses, including HIV, hepatitis C, Coronavirus and Japanese encephalitis. Regarding HIV, this protein has a combination of (i) higher potency than other lectins, (ii) excellent pre-clinical properties including low/no toxicity and non-activating to a variety of cell types, (iii) inexpensive production in gram quantities, and (iv) synergy when combined with antibodies and a variety of other lectins. It binds tightly to the HIV gp120 through high-mannose saccharides on the gp120 surface and potently inhibits viral infection. Grft has been shown to be a tight dimer, but the role of the dimer in function has not been fully explored. To investigate the role of the Grft dimer in anti-HIV function, an obligate dimer was designed by expressing the protein with a peptide linker between the two subunits. This "Grft-linker-Grft" is a folded protein dimer, and NMR shows it is nearly identical in structural properties with the wild type protein. A "one-armed" obligate dimer was also designed, with one of the subunits mutated to eliminate its carbohydrate binding ability while the other subunit remained intact. While both constructed dimers retained the ability to bind gp120 and the viral surface, Grft-linker-Grft-OneArm was 84 to 1010 fold less able to inhibit HIV compared to wild type Grft, while Grft-linker-Grft had near wild type potency. Furthermore, while wild type Grft demonstrated the ability to alter the structure of gp120 by exposing the CD4 binding site, Grft-linker-Grft-OneArm largely lost this ability. These experiments provide evidence that the dimer form of Grft is critical to the function of this protein in HIV inhibition, and suggest that the role of the dimer may in part be to affect the structure of gp120 as well as to allow higher avidity for the lectin. Ongoing experiments will further investigate the conformational change induced by Grft and its correlation to anti-HIV activity.

105 Human Milk Sialylated Galactosides Inhibited Enterovirus 71 and A(H1N1) 2009 Influenza Infection in Respiratory and Gastrointestinal Cell Lines

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[1]University of North Carolina, Chapel Hill, USA, [2]Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

BACKGROUND**:** Many viruses recognize specific sugar residues, such as sialylated glycosides, as the infection receptors. Human milk contains many sialylated oligoglycosides which may block viral infections to respiratory and gastrointestinal tract. We postulated that sialic acid-linked alpha 2,3 galactosides (SA-α2,3Gal) and SA-α2,6Galfrom human milk could inhibit infection of enterovirus 71 (EV71) or novel A(H1N1) 2009 influenza. We have previously shown that SA-α2,6Gal and SA-α2,3Gal from human milk specifically inhibited EV71 infection to DLD-1 intestinal cells (*Virol J.* 2009, 6:141). This study has been extended to investigate whether SA-α2,6Gal and/or SA-α2,3Gal from human milk inhibited novel A(H1N1) 2009 influenza infection to A549 respiratory and DLD-1 intestinal cell lines differently. RESULTS: EV71 infected both A549 and DLD-1 cells, but A(H1N1) 2009 influenza mainly infected A549 cells. Depletion of O-linked glycan, but not N-linked glycan, significantly decreased EV71 infection. Inhibition of N-glycan formation suppressed A(H1N1) 2009 influenza infection of DLD-1 cells. This suggests that SA-linked O-glycans and N-glycans on respirato-intestinal cells were respectively responsible for EV71 and A(H1N1) 2009 influenza infections. Both SA-α,3Gal and SA-α,6Gal from human milk significantly inhibited EV71 infection of DLD-1 and A549 cells. Only SA-α,6Gal from human milk partly inhibited novel A(H1N1) influenza infection to A549 cells. SUMMARY AND IMPLICATIONS: O-linked and N-linked glycans on respirato-intestinal cells were respectively responsible for the virus entry of EV71 and A(H1N1) 2009 influenza infections. Sialylated galactosides from human milk could inhibit A(H1N1) 2009 influenza and EV71 infection of respiratory and/or gastrointestinal cells, suggesting food with SA-linked galactosides might protect respiratory and/or gastrointestinal cells from A(H1N1) 2009 influenza and EV71 infections.

106 Hepatitis B Virus Replication Kinetics Patterns and Their Impact on Screening Anti-HBV Therapeutic Agents in HepG2.2.15 and AD38 Cell Lines

Yong-Yuan Zhang, Robert W Buckheit, Jr.
ImQuest BioSciences, Inc., Frederick, USA

Cell-based assays for evaluating anti-HBV therapeutic agents primarily utilize stably HBV-transfected cell lines. The optimization requirements for use of these cell based assays have not been clearly defined due to a lack of understanding and quantification of the kinetics of HBV DNA replication in the target cell lines. Therapeutic products which target HBV RTonly impact newly synthesized HBV DNA molecules robust HBV DNA replication is required to fully evaluate the efficacy of these anti-HBV agents. HBV DNA replication is determined by the quantity of pregenomic RNA (pgRNA) in the HBV DNA stably transfected cell lines. Defining the pgRNA levels during replication is critical to effectively using these cell lines for screening of compounds. We have investigated the kinetics of pgRNA, intra- and extracellular HBV DNA in both AD38 and HepG2.2.15 cell lines and evaluated the effect of active antiviral agents on these products. Two patterns of HBV DNA replication were detected in HepG2.2.15 cells: (1) pgRNA levels remain steady, leading to little or equilibrium HBV DNA replication. In this pattern,, 90-99% of the HBV DNA is retained intracellularly with highly inefficient secretion of virus into extracellular medium. Such replication is not suitable for testing anti-HBV therapeutic agents since most compounds appear inactive; (2) HBV DNA replication demonstrates a greater than 10-fold increase during the time course of the antiviral assay accompanied by relatively low intracellular HBV DNA retention. This replication pattern allows inhibition of HBV DNA replication by anti-HBV therapeutics. AD38 cells show increased pgRNA levels on a daily basis, leading to a 100-fold increase in HBV DNA replication. The production of HBV DNA is effectively inhibited by positive control compounds and thus represents a more robust system for antiviral compound evaluations although increasing quantities of HBV DNA may be delayed, requiring longer assays to achieve high levels of HBV replication. Additionally it should be noted that there is no uniform replication kinetics pattern for HBV DNA replication even in the same cell line. Thus, for best results, both intra- and extracellular HBV DNA levels must be quantified.

107 Human Serum Contains Homologous Protein(S) to Those Found in Duck Serum That Enhance Duck Hepatitis B Virus cccDNA Synthesis: Implications for the Treatment of HBV

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ImQuest BioSciences, Inc., Frederick, USA

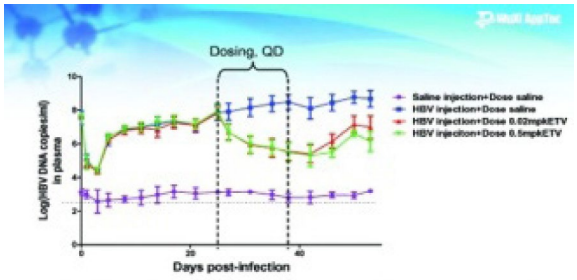
The efficient synthesis of DHBV cccDNA in DHBV-infected primary hepatocytes is critically dependent on the presence of a specific duck serum protein(s). Decay of this protein(s) in duck serum reduced cccDNA synthesis by as much as 100-fold. The DHBV replication pathway, including cccDNA conversion, faithfully reflects HBV replication, implying similar/identical mechanisms are employed by both DHBV and HBV for replication. Experimental evidence shows that host factors required for virus replication, even from different species, can function well in facilitating DHBV replication. For instance, both DHBV and HBV pgRNA molecules interact with DNA polymerase molecules to form the ribonucleoprotein complex for initiating reverse transcription. Cellular factor Hsp90 and its partner p23 are required to facilitate such interaction. Surprisingly, the use of rabbit Hsp90 and human p23 as the rabbit Hsp90 partner facilitates efficient protein-protein interactions in the DHBV system. These results prompted us to evaluate the presence of a homolog protein(s) in human serum that would successfully and efficiently facilitate DHBV cccDNA synthesis. We investigated this issue by adding fresh human serum to the duck primary hepatocyte culture upon inoculation with the DHBV positive serum in which the required serum protein(s) were eliminated by freeze-thawing (as previously described). DHBV cccDNA was evaluated from the infected cells at day 7 post infection. Three of seven tested human sera samples tested increased cccDNA synthesis by 3-5-fold, accompanied by a corresponding reduction in the ratio of intracellular rcDNA and cccDNA (up to 10 fold), suggesting that more rcDNA was transported to nuclei for cccDNA conversion following the addition of human serum to the infected primary duck hepatocytes. Our results would suggest that an important factor controlling HBV cccDNA synthesis is shared by human and ducks and that the levels of cccDNA-enhancing serum protein(s) are somewhat variable from person to person. Quantification of the huma serum protein(s) may allow better understanding of potential HBV pathogenesis and outcomes and yield an important antiviral target linked to control of cellular cccDNA levels.

108 FRG™ Mice with Humanized Livers as a Robust Animal Model for HBV Drug Discovery

Qiong Zhou[1], Qian Chen[1], Qiugang Lei[1], Xiaoyu Zhao[1], Elizabeth/M Wilson[2], John Bial[2], Henry Lu[1], Xinsheng Chen[1]

[1]*WuXi AppTec (Shanghai), WaiGaoQiao Free Trade Zone, China,* [2]*Yecuris Corporation, P.O. Box 4645 , USA*

A robust animal model for human hepatitis B virus (HBV) is needed for the discovery of novel drugs for treatment of patients chronically infected by HBV. FRG™ ($Fah^{-/-}$/$Rag2^{-/-}$/$Il2rg^{-/-}$) triple ko mice are deficient in fumarylacetoacetate hydrolase (FAH), which creates a drug controllable mouse liver ablation mechanism. The animals are further modified to be immunodeficient, but are otherwise healthy. Human hepatocytes, injected intrasplenically into the mice, can efficiently replicate to form a chimeric liver with up to 98% human cell repopulation. The human hepatocyte repopulation can last for the life of the animal irrespective of the age of the mice or source of the donor of hepatocytes. Here we show that FRG™ KO mice on the C57B/6 background carrying human hepatocytes (FRGC mice) were susceptible to the infection with patient HBV sera. HBV virion DNA, HBsAg and HBeAg in plasma and viral replication intermediates, transcripts and cccDNA in liver tissues were detected. The HBV viral titer in plasma was as high as 10^8~10^9 copies/mL, providing a robust window for antiviral efficacy testing. The FRGC mice were infected with HBV patient serum, followed by daily dosing of Entecavir (ETV) or vehicle for two weeks when HBV virimia reached steady state. Upon ETV treatment, plasma HBV DNA titer decreased by ~3 orders of magnitude in each of the animals. At 2 weeks after the last ETV dosing, 10-fold rebound of HBV viremia was observed. In summary we have established a robust model of HBV infection for evaluation of antiviral efficacy for HBV drug candidates targeting the entire HBV replication cycle, including the viral cccDNA.



Data Fig. HBV infection with patient HBV plasma and anti-HBV efficacy of entecavir in the FRGC mouse model Day 0, the FRGC mice were injected with 10^5 GE HBV DNA or normal saline through tail veins.

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POSTER SESSION 2: HERPESVIRUSES, POXVIRUSES, ENTEROVIRUSES, EMERGING VIRUSES, OTHER ANTIVIRAL AGENTS AND MEDICINAL CHEMISTRY

4:30 pm – 6:30 pm

GRAND BALLROOM A and GRAND FOYER

124 Nano-Effect of Clay minerals on Human Papillomavirus-Warts

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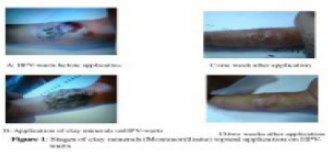
University of Mosul, Mosul, Iraq

Clay minerals consist 16% of earth minerals and its nano sheet-like structure make it a useful materials used in many medical applications due to its adsorption or absorption of different organic materials. The aim of this study was to test the application of clay minerals as nano antiviral agent against human papilloma virus (HPV)-warts for the first time. Clay minerals structures, major elements and trace elements were determined by X-ray, flame photometer and atomic absorption analysis respectively. Four types of clay minerals were topically applied against HPV-warts. Montmorillinite showed the best effect on HPV-warts with 100% of warts recovery. Mixture of Kaolinite, Montmorillinite, and Illite also gave high effect with 80% of warts recovery. Kaolinite alone showed moderate effect by recovering 66.6% of warts whereas Kaolinite with organic materials showed low effect with 22.2% of warts recovery. Further studies is needed to test clay minerals against others cancerous types of HPV which cause genital and cervical cancerous warts in a high ratio scattered all over the world.



125 Genotypic and Phenotypic Herpes Simplex Virus Type 2 (HSV-2) Dynamics of Drug-Resistant Mutations During Antiviral Therapy in an Hematopoietic Stem Cell Transplant (HSCT) Recipient

Graciela Andrei[1], Florence Morfin[2], André Boibieux[2], Sophie Ducastelle[2], Sarah Gillemot[1], Ghislain Opdenakker[1], Robert Snoeck[1]

[1]*Rega Institute for Medical Research, KU Leuven, Belgium,* [2]*Hospices Civils de Lyon, Lyon, France*

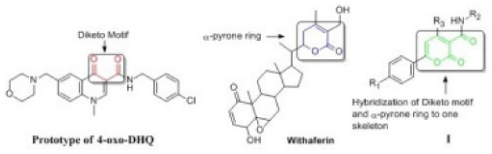
Herpesviruses continue to cause significant morbidity and mortality in HSCT recipients despite the availability of effective therapies and infections caused by drug-resistant isolates are an emerging concern among these patients. Understanding evolutionary aspects of HSV infection is crucial for designing therapeutic strategies. We report here the characterization of 11 sequential HSV-2 isolates recovered from a HSCT patient (treated with acyclovir and foscavir) who suffered from a primary HSV-2 infection with involvement of various body sites. A total of 11 isolates recovered during a period of two months from diverse body locations were analyzed phenotypically (drug-resistance profiling) and genotypically (sequencing of the viral thymidine kinase and DNA polymerase genes by the Sanger method), and showed that most of the isolates were mixed populations of different DNA polymerase mutants. Because of the heterogeneity of the viral isolates, several plaque-purified viruses from each isolate were genotypically characterized allowing the identification of known (R628C, A724V, S729N) and new (K533E, A606V, C625R, S725G, I731F, Q732R, M789T/K, Y823C, V842M, R847C, F923L, T934A, R964H) DNA polymerase amino acid changes associated with resistance to acyclovir, foscavir and PME derivatives. As we identified more drug-associated mutations among the plaque purified viruses than observed in the original isolates, deep-sequencing analysis was performed, showing that the proportion of the drug-resistant variants varied from 1- 95%, depending on the isolate. In contrast, the original viral isolate showed a unique wild-type DNA polymerase variant. Some of the DNA polymerase mutants (A606V, S729N, Y823C, V842M, and R847C) appeared to have a reduced fitness because: i) the percentage of variants identified by deep-sequencing did not correlate with the proportion of mutants found by plaque-purification and ii) competitive in-vitro fitness studies showed that they were impaired in growth. The existence of minor drug-resistant variants should be taken into account in the therapy of HSV infections.

126 A Skeletal Hybridization Approach to Generate Novel α-Pyrone Analogs as Anti-HSV Agents

Chandralata Bal[1], Srinivas Karampuri[1], Paromita Bag[2], Debprasad Chattopadhyay[2], Ashoke Sharon[1]

[1]*Birla Institute of Technology, Mesra, Ranchi, India,* [2]*ICMR Virus Unit, Beliaghata, Kolkata, India*

Replication of DNA in Herpes Simplex Virus (HSV) is mediated through its DNA polymerase enzyme. A number of non-nucleoside inhibitors (NNI) focusing allosteric domains of the DNA polymerase are currently under research and development. We found 4-oxo-dihydroquinolines (4-oxo-DHQs) and Withaferin A (WA), a naturally occurring C28-steroidal lactone from Indian ginseng as interesting class of molecules as the starting point of our ongoing anti-HSV drug discovery project. 4-oxo-DHQs have shown high specificity index in inhibiting DNA polymerases of herpesviridae family because unrelated DNA and RNA viruses were not susceptible to their inhibitory effect. WA exerts its inhibitory effect via interaction with a viral DNA polymerase site that is less important for the binding of deoxynucleoside triphosphates and could be able to act on resistant viruses also. Close keto groups in 4-oxo-DHQ and α-pyrone ring of WA are the major pharmacophoric motifs. Therefore, we were interested to introduce both these pharmacophores into a sinlge skeleton (I). We had analysed the designed molecules (I) for their binding to HSV DNA polymerase in the palm region using molecular modeling. The semi-empirical binding score and binding mode were considered as preliminary basis for the screening of the analogs for synthesis. The Anti-HSV activities of synthesized compounds were evaluated by MTT assay on African green monkey kidney cell (Vero cells, ATCC, Manassas, VA, USA). One of the synthesized compounds has EC_{50} of 9.82 µg/ml and CC_{50} of 151.8 µg/ml leading to selelective index of 15.42. (Authors thanks to DBT, India and DST India for Reseach Grant)



127 Inhibition of SARS-CoV by Thiazolidin-4-ones

Dale L Barnard[1], Yohichi Kumaki[1], Kevin Bailey[1], Donald F Smee[1], John D Morrey[1], Roman Lesyk[2]
[1]*Utah State University, Logan, USA,* [2]*Danylo Halytsky Lviv National Medical University, Lviv, Ukrenia*

Thiazolidin-4-ones are a class of compounds with a broad spectrum of biological activities, which include antibacterial, antitumor, antihistaminic, anti-inflammatory, and anticonvulsant activities. We evaluated a number of derivatives in this class of compounds and found that **3-(4-diethylaminophenyl)-2-(4-dimethylaminophenyl)-4-thiazolidinone** had good, selective activity against SARS-coronaviruses. In a cytopathic effect reduction assay (CPE), the compound inhibited SARS-CoV strain Urbani replication with an EC50 of 3.1 ± 0.2 µg/ml with a selective index of >32.5 ± 2.1. In a neutral red dye uptake assay (NR) the EC50 was 5.9 ± 3.7 µg/ml and the selective index was >21 ± 14. Cytotoxicity was not observed even at concentrations as high as 100 µg/ml. This moderate antiviral activity was validated in a virus yield reduction assay in which the EC90 was equal to 4.2 µg/ml. The 4-thiazolidinone derivative also similarly inhibited other strains of SARS-CoV, including the Tor-2, Frankfurt-1, and CuHK-1 strains with EC50 values ranging from 7.5 -16 µg/ml in CPE assays and from 16-24 µg/ml in NR assays. It also inhibited Junin virus with an EC50 value of 12 µg/ml in a CPE assay and an EC50 value of 5.7 in a NR assay. Otherwise, the inhibitory spectrum appeared to be restricted to inhibition of SARS-CoV replication since it did not inhibit paramyxoviruses, orthomyxoviruses, picornaviruses, adenovirus, bunyaviruses, or viruses from the *Flaviviridae*, including Dengue virus 2 and Japanese encephalitis virus. The compound was also initially evaluated in a SARS-CoV lethal BALB/c mouse model in which the compound was administered intraperitoneally bid X 5 at 32, 10, 1, and 0.1 mg/kg/d in a 1% DMSO PBS vehicle. Although not toxic at any dose administered to mice, the 4-thiazolidinone derivative did not protect mice against death due to the virus infection. Since the compound was difficult to keep in suspension, the compound was likely not bioavailable in this vehicle. However, it may be that an alternative vehicle may vehicle may render the compound bioavailable to promote efficacy in this mouse model. This work was supported, in part, by Task Order B05, HHSN27200002, from the United States National Institutes of Health.

128 Defining the Required Tissue Concentration of an Antiviral Agent to Totally Suppress Infection, Replication, and Transmission

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We have described a microbicide transmission sterilization assay (MTSA) to quantify the concentration of a topical microbicide product required to suppress the sexual transmission of HIV-1. Additional development of the MTSA has resulted in the hypothesis that the MTSA-defined sterilizing concentration may approximate the required tissue concentration of an active agent at the site of infection to assure efficacy. Data from *in vitro* and *ex vivo* assays support the concept that a microbicide product must attain concentrations that are 100-1000-fold higher than the *in vitro* defined EC_{50} concentration in order to be effective. The tissue concentration achieved is highly dependent on the chemical nature of the product and the formulation employed for delivery to the tissue. Biological evaluations of a variety of potential microbicide products have shown that analogs of highly active products have varying sterilizing concentrations despite possessing similar inhibitory concentrations and solubility. In addition, differences in sterilizing concentrations appear within products of the same inhibitory classes, suggesting that the definition of the sterilizing concentration may be a critically important component of preclinical development algorithms. Biological evaluations at sterilizing concentrations support the notion that the results of the MTSA are not merely due to tissue culture artifacts. The sterilizing concentration can be determined within days of the initiation of an acute infection of human target cells. Our *in vitro* and *ex vivo* definition of sterilizing concentrations has continued to evolve to include evaluation of the ability of test compounds or combinations of compounds to sterilize cultures and has resulted in the expansion of the MTSA to evaluate products active against other sexually transmitted infections (HSV-1 and HSV-2), as well as viruses such as influenza. We believe the sterilization assay thus may be an early means of prioritizing compound development based on the relative ability of a compound to prevent the replication of viruses in target cells and tissues as well as to prevent the spread of virus from initially infected cells to surrounding target cells in tissue.

129 The ImQuest SUCCESS Drug Development Platform: Enhancing Successful Drug Development Opportunities

Robert W Buckheit, Jr., Karen W Buckheit, Christian Furlan-Freguia, Anthony Ham, Tracy L Hartman, Mansoora Khaliq, Todd B Parsley, Yong-Yuan Zhang
ImQuest BioSciences, Inc., Frederick, USA

Successful drug development is dependent on a variety of complex variables that are difficult to assess poor drugs often advance to clinical testing that should have been deprioritized during preclinical evaluation. Thus, rising costs associated with drug development result from the need to finance drug development failures. We have developed a platform of assays that allow the rapid delineation of critical preclinical properties of therapeutic and prevention products. The ImQuest***SUCCESS*** platform provides the strongest possible foundation of preclinical data, allowing rationale prioritization of compounds for continued development based on their potential for clinical success. The ***SUCCESS*** platform includes efficacy, safety, and pharmaceutical properties of a potential product, all of which may be evaluated in a cost-effective manner prior to significant investment in advanced preclinical and clinical development. The ImQuest***SUCCESS*** platform may be used to evaluate the development potential of agents targeting infectious viruses, bacteria, and fungi. Efficacy assays define the therapeutic index of products in the biological matrices in which they act using *in vitro* and *ex vivo* assays that measure the range and mechanism of action, resistance selection, and activity in combination with other products. The platform defines the tissue concentration of a product required at the site of infection to sterilize infection. The ImQuest***SUCCESS*** platform also provides critical measurements of *in vitro* and *ex vivo* cytotoxity, compound permeation and compound metabolism, as well as *in vitro* and *ex vivo* pharmacokinetic and pharmacodynamics. Finally, ImQuest***SUCCESS*** evaluates important pharmaceutical properties of a test compound critical to the formulation and delivery of a compound with optimal PK/PD and attained tissue sterilizing concentrations. The ImQuest***SUCCESS*** platform thus provides a foundation of essential efficacy, safety and pharmaceutical property data that may be utilized to rationalize continued financial and manpower investments in products with enhanced potential for clinical success.

130 X-ray Studies on the Mechanism of Inhibition of Foot-and-Mouth Disease Virus VPg-1 by FUTP

María-José Camarasa[1], Gloria Fernández-Cureses[1], Sonia De Castro[1], Cristina Ferrer-Orta[2], Nuria Verdaguer[2], Esteban Domingo[3]
[1]Instituto de Química Médica (IQM-CSIC), Madrid, Spain, [2]Institut de Biología Molecular (IBMB-CSIC), Barcelona, Spain, [3]Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC), Madrid, Spain

The foot-and-mouth disease virus (FMDV) is the cause of a highly contagious disease concerning cloven-hoofed animals. VPg protein Initiates viral RNA synthesis, the first step being the linkage of UMP to the Tyr3 hydroxyl group of the VPg protein. Virally encoded RNA-dependent RNA polymerase (3D) requires the uridilylated form VPg to act as the primer for both, positive- and negative-strand synthesis. FUTP is a potent competitive inhibitor of VPg-1 uridylation. By peptide analysis a VPg fragment containing FUMP covalently attached to Tyr has been identified. However, the molecular basis of this phenomeno is still unknown.

We describe the synthesis and X-ray studies of two models of VPg-1 that contain U or FU in a 15 mer peptide linked through the hydroxyl group of Tyr3. X-ray structure of 3D-pol FMDV/VPg-FU showed a significant conformational change at the b9-a11 loop, protruding into the active site of the polymerase, thus, blocking the access of the template and of the incoming nucleotides.

131 Targeting CVB3 3A Protein: A Virtual Screening Approach

Michela Cancellieri[1], Pieter Leyssen[2], Rachel Ulferts[3], Frank van Kuppeveld[3], Johan Neyts[2], Andrea Brancale[1]
[1]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, [2]Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium, [3]Department of Infectious Diseases & Immunology, Utrecht University, Utrecht, Netherlands

Coxsackievirus B3 (CVB3) is a human pathogen that belongs to the Enterovirus genus, a member of the *Picornaviridae* family. This virus, which has a worldwide distribution, is responsible for wild but also more serious diseases, including meningitis, encephalitis, pancreatitis and viral myocarditis with a pronounced incidence in children and young adults. The lack of a specific therapy against the virus makes its mechanism of replication an interesting area for the development of antivirals and nonstructural proteins represents one of the most promising targets. Of particular interest is the small 3A protein, which is able to form homodimers and plays important roles in genome replication and inhibition of intracellular protein transport. Dimerization has been reported to be essential for the biological functions of the protein, specifically hydrophobic interactions between the two monomers and an intermolecular salt bridge. A homology model of the CVB3 3A protein, using structural information from the closely related poliovirus 3A protein, was developed and used in a virtual screening simulation, targeting the key residues involved in dimerization. One compound was identified to inhibit viral replication in infected cells at high μM concentration. To further investigate the potential biological activity of this molecule, a series of close analogues were also evaluated for antiviral activity in cell-based assays. In this presentation, we will discuss the preliminary results obtained, the biological data and their implication for further SAR studies to be conducted on this series of compounds.

132 Efficacy of N-Methanocarbathymidine (N-MCT) Against Herpes Simplex Virus Type 2 in a Genital Guinea Pig Model

Rhonda Cardin[1], Fernando Bravo[1], Julie Earwood[1], Jennifer Clark[1], Derek Pullum[1], Robert Glazer[2], Aquilur Rahman[3], David Bernstein[1]
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N-MCT has demonstrated potent activity against HSV-1 and HSV-2 infections in mouse and neonatal guinea pig models. In this study, the efficacy of N-MCT was evaluated in the guinea pig model of genital HSV-2 infection, as this model mimics many aspects of human disease. N-MCT was administered i.p. twice daily beginning at 24 hours post vaginal HSV-2 inoculation and continued for 7 days at 25 or 50 mg/kg and compared to 100 mg/kg acyclovir (ACV) or vehicle. The severity of acute disease (daily score 0-4), acute vaginal virus replication, and recurrent disease were evaluated (n=12/group). At 63 dpi, the latent virus load was determined in the dorsal root ganglion (DRG) and spinal cord by qRT-PCR. Drug levels were measured 1 hr after dosing on days 3 and 7. N-MCT reduced the severity of acute disease: no animals receiving 50 mg/kg N-MCT and only 4 animals receiving 25 mg/kg N-MCT developed acute disease compared to 8 and 10 animals receiving ACV or vehicle, respectively ($P< 0.01$). Similarly, vaginal replication was more rapidly reduced by N-MCT compared to ACV. Cumulative recurrent lesion scores were also significantly reduced to 1.9 and 2.8 in animals receiving 25 or 50 mg/kg N-MCT versus 4.9 and 7.2 in animals receiving ACV or vehicle. HSV-2 was detected in the spinal cord and DRG in 75% and 92% of animals receiving ACV or vehicle but was detected in < 33% of animals receiving 50 or 25 mg/kg N-MCT ($P< 0.01$ vs. ACV). Plasma levels of N-MCT at days 3 or 7 were similar, with an average of 4.6 and 8.3 μg/ml at 25 mg/kg and 50 mg/kg N-MCT, respectively. These studies demonstrate that N-MCT is more effective than ACV in reducing acute and recurrent disease as well as latent viral load following vaginal HSV-2 infection. Studies are currently underway to determine the efficacy of N-MCT in reducing recurrent lesions when administered therapeutically in the genital herpes model. [supported by NIH contract HHSN272201000008I]

133 Design, Synthesis and Evaluation of Potent Trisialic Acid (TSA) Compounds as Inhibitors of Ocular Adenovirus Type 37

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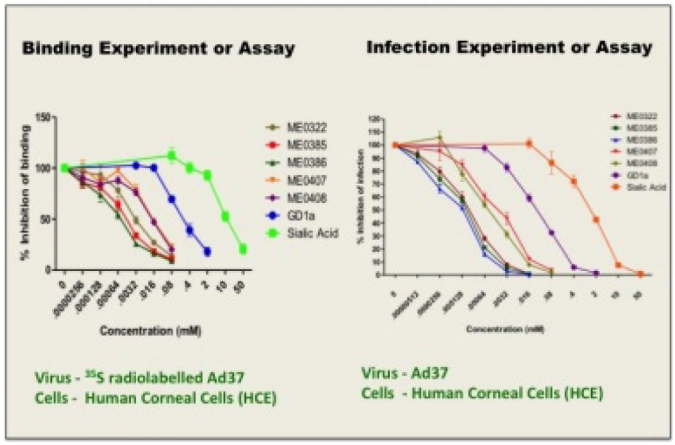
There are more than 60 human Adenovirus (Ad) types have been classified into seven species (A-G) and causes infection to wide range of species and tissues. Group D Ad contains more than 30 members and commonly causes ocular infections. The most occurring ocular infection is Epidemic keratoconjunctivitis (EKC), mainly caused by highly contagious Ad8, 19 and 37 which develops symptoms such as pain, edema, lacrimation and decreased vision for few month. Worldwide 10-25 million people suffers from severe EKC, caused by group D Ads including Ad37. Since there are no vaccines or antiviral drugs available, identification of novel targets for antiviral treatment is highly desireable. Ads are non enveloped viruses with double stranded DNA genome surrounded by a capsid made up of hexon, penton and fiber protein. The terminal, trimeric knob domain of the capsid fiber protein, binds to cellular receptors. Our group has already shown that sialylated proteins serve as cellular receptors for group D Ads. This study opened the doors to new approach for structure based drug design. Here, we have designed and synthesized compounds having three terminal intact or modified sialic acid linked through spacers. The length of these spacers corresponds to distance between two sialic acid binding sites in the Ad fiber knob. Antiviral evaluation of these TSA compounds were carried out by molecular interaction studies through X-Ray crystallography and surface plasmon resonance as well as by performing *in vitro* binding and infection assays. Here, we show that two out of five TSA compounds (ME0385 and ME0386) are efficient inhibitors of Ad37 binding to and infection of human ocular cells.



134 RNA Competition Screening Assays in Dengue Target-based Drug Discovery

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Dengue virus (DENV) is the most prevalent mosquito-borne viral pathogen in humans, with several million viral infections occurring annually, for which no effective therapy currently exists. In the target-based approach, large chemical compound libraries are routinely screened through selected enzymatic activity assays. Vital to the often laborious evaluation of chemical compound hits in large high-throughput screens is the application of various orthogonal and selectivity screening assays to focus medicinal chemistry efforts on specific chemical scaffolds in which to begin structure-activity relationship (SAR) analysis. Here we present the development of two simple, high-throughput, highly-sensitive RNA competitive binding assays using commercially available fluorescent nucleic acid binding dyes to eliminate compounds that bind non-specifically to the RNA template in a dengue virus RNA-dependent RNA polymerase(RdRp) enzymatic assay. These two assays are suitable for use as counter-screens in hit-to-lead finding programs involving DNA or RNA polymerases.

135 The Highly Selective Inhibition of Epstein-Barr Virus Replication by KAY-02-41 is Dependent on the Virus Thymidine Kinase

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Several 4'-thiopyrimidine nucleoside derivatives were shown to possess inhibitory activity against herpesviruses. The 1'-methyl analog of 4'-thiothymidine (KAY-02-41) was formerly demonstrated to exhibit moderate antiviral activity against herpes simplex virus type 1 (HSV-1). We evaluated the potency of KAY-02-41 *in vitro* against a wide spectrum of herpesviruses by determination of the 50% effective concentrations (EC_{50}) against these viruses, and the 50% cytotoxic effects (CC_{50}) in their respective cell lines. KAY-02-41 was found to be highly active against Epstein-Barr virus (EBV) replication in P3HR-1 cells (EC_{50} = 0.7 μM) and showed a therapeutic selectivity of 224. The compound showed moderate activity against HSV-1, HSV-2 and murine gammaherpesvirus 68 (MHV-68) and weakly inhibited varicella zoster virus, herpesvirus saimiri (HVS) and rhesus rhadinovirus replication. No inhibition of human cytomegalovirus and Kaposi's sarcoma-associated herpesvirus replication was observed. KAY-02-41 was evaluated in a mouse model of γ-herpesvirus infection using MHV-68. The drug was administered intraperitoneally at 50 mg/kg for 5 consecutive days. At 6 days p.i., KAY-02-41 treatment reduced the viral DNA load and virus gene expression in the lungs ($p< 0.01$). At day 12 p.i., the compound still exerted a moderate inhibitory effect in the lungs of infected mice, but not in the mediastinal lymph nodes and spleen. Interestingly, another analog showed superior potency in this *in vivo* model. Mutant HSV-1, MHV-68 and HVS strains selected *in vitro* for resistance to KAY-02-41, harbored mutations in the viral thymidine kinase (TK). Phenotypic characterization revealed that these mutations conferred low-resistance to KAY-02-41, whereas other TK-dependent drugs showed high level of resistance. Enzymatic studies further demonstrated that KAY-02-41 was a good substrate of HSV-1 TK, but not for the cellular TK-1 and TK-2. Our studies showed that KAY-02-41 requires herpesvirus TK for its activation, and may be of interest in the treatment of EBV-associated diseases.

136 Altered Cyclic HPMPC Metabolism in Induced P3HR-1 cells Accounts for its Reduced Antiviral Activity against Epstein-Barr Virus

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We previously reported that cyclic forms of acyclic nucleoside phosphonates (ANPs) showed 10- to 50-fold diminished antiviral activity against Epstein-Barr (EBV) replication in P3HR-1 cells, but not in Akata cells, compared to their non-cyclic forms. We therefore investigated the metabolism of HPMPC and cyclic HPMPC (cHPMPC) in both cell lines, as well as the potential involvement of cyclic cAMP (cAMP) in the reduced anti-EBV activity of cyclic forms of ANPs. P3HR-1 and Akata cells were induced to the lytic cycle and incubated with 10μM of [^{3}H]-HPMPC or [^{3}H]-cHPMPC. Metabolite concentrations were analyzed after 24, 72 and 120h. Total radioactivity in P3HR-1 cells incubated with [^{3}H]-HPMPC was found to be 6-fold higher compared to Akata cells, while only a 2.5 fold increase in radioactivity was noted after incubation with [^{3}H]-cHPMPC. Also, metabolic conversion of cHPMPC to HPMPC was remarkably less efficient in P3HR-1 cells than in Akata cells. In P3HR-1 cells, incorporation of radiolabel into DNA was 3- to 4-fold lower after incubation with [^{3}H]-cHPMPC compared to [^{3}H]-HPMPC; this difference was not seen in Akata cells. In contrast, the metabolism of both compounds was similar in latently-infected P3HR-1 and Akata cells. Levels of cAMP were determined by Elisa in latently-infected and induced P3HR-1 and Akata cells at 24, 72 and 120h. Only P3HR-1 cells showed reduced cAMP concentrations after induction to the lytic cycle, from approximately 60 pmol per 10^6 cells in latently-infected cells to approximately 15 pmol per 10^6 cells in induced cells ($p=0.008$). Previous studies have reported the role of cAMP in the maintenance of EBV latency. Therefore, induction of the EBV lytic cycle in P3HR-1 cells might induce pathways that result in elimination of cAMP. This may involve enhanced cellular export of the cyclic nucleotide and/or hydrolysis of cAMP by phosphodiesterases. Thus, induced P3HR-1 cells showed an enhanced efflux of cHPMPC. Alternatively, cAMP could potentially compete with cHPMPC for hydrolysis by the 2',3'-cyclic-nucleotide 3'-phosphodiesterase. Additional experiments are being performed to confirm the role of cAMP in the reduced activity of cyclic forms of ANPs in induced P3HR-1 cells.

137 High Resolution Iodixanol Gradients Reveal Aberrant Capsid Formation Upon Small Molecule Blockade of Host Factor Targets Involved in Capsid Assembly

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New approaches to biology often require development of novel methods or refinement of old ones in ways that make previously unappreciated relationships more readily apparent. We have developed cell-free protein synthesis (CFPS) systems that are believed to recreate the pathway of capsid assembly *de novo,* and have applied these systems across multiple viral families to identify small molecules with potent antiviral activity against infectious virus in cell culture. The host targets of these small molecules are novel and unconventional multiprotein complexes, making target identification and mechanism of action deconvolution particularly challenging. To fully exploit the potential of these host targets for the development of antiviral therapeutics, a better understanding of mechanism of action of these novel small molecules is required. Rather than the simpler phenotype of blockade of release of virus as might have been expected, we have observed that aberrant capsids, as scored by release of non-infectious viral particles from drug-treated cells, are a common but complex phenotype of the action of these compounds. A simple method by which to score viral capsids as aberrant would greatly facilitate the application of this approach. Here we use variations on the theme of the classical method of equilibrium cesium chloride gradient centrifugation, using iodixanol as the media, to provide a simple means of scoring capsid aberrancy. Together with evidence that the equilibrium densities of cell-free assembled capsid structures are comparable to the diversity of densities observed for virus released from cells, this approach provides a valuable new tool for advancement of host-targeted small molecule therapeutics.

138 L-BHDU Requires VZV TK and Prevents Virus Replication by Competition with the Pyrimidine Biosynthesis Pathways

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New antiviral drugs for varicella-zoster virus (VZV) with increased potency are needed, especially to treat herpes zoster. The bromovinyl uracil nucleoside, L-BHDU, is effective against VZV in culture and in a mouse model. The active form is hypothesized to be L-BHDU monophosphate (MP), which is the only form detected in VZV-infected cells, although the mechanism of action is unknown. Resistant strains of pOKA VZV-BAC-Luc and VZV-Ellen were isolated against L-BHDU. The amino acid substitutions G22R and R130Q were mapped to the active site of the viral thymidine kinase (TK). We found cross-resistance to ACV and brivudin (BVdU) but not Foscarnet (PFA), confirming that L-BHDU activity is dependent on VZV TK. To address whether L-BHDU-MP interfered with purine or pyrimidine synthesis, VZV-infected cells were treated with drug in the presence of 100-fold excess nucleosides. Addition of pyrimidines (thymidine or uridine) restored wild type VZV replication while purines (adenosine, guanosine or inosine) and the de novo pathway intermediates (orotate and dihydroorotate) did not. To investigate whether L-BHDU-MP inhibited pyrimidine biosynthesis, HFF cells stably expressing VZV TK were treated with the drug and cell growth was measured. L-BHDU was nontoxic and HFF-TK cells grew normally, indicating that L-BHDU-MP did not affect cellular pyrimidine biosynthesis, which is unlike BVdU that blocks cellular thymidylate synthase (TS). To investigate whether L-BHDU-MP inhibited viral TS (ORF13), the mutant virus rOka13S (stop codon in ORF13) was grown in contact inhibited HFFs, which have negligible cellular TS. In the presence of L-BHDU, thymidine but not uridine restored rOka13S replication. The current working model of L-BHDU antiviral mechanism is that phosphorylation by VZV TK produces L-BHDU-MP that competitively inhibits viral TS, thereby blocking the de novo pyrimidine biosynthesis pathway. HPLC studies to investigate the effects of L-BHDU on the pyrimidine salvage pathway are in progress.

139 Repopulation of Ganciclovir-Resistant Cytomegalovirus by Wild Type Virus

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We report a patient in whom ganciclovir-resistant CMV was replaced by wild type virus after discontinuation of ganciclovir/valganciclovir and review other similar cases. Repopulation by wild type virus may occur soon after discontinuation and may be fostered by discontinuing ganciclovir altogether rather than continuing it in combination with foscarnet when treating patients with ganciclovir-resistant CMV disease.

140 N-Alkyldeoxynojirimycin Derivatives with Novel Terminal Tertiary Amide substitution Active Against Multiple Hemorrhagic Fever Viruses

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Hemorrhagic fever viruses contain different RNA genomes, but they are all enveloped with glycosylated proteins, which requires processing by endoplasmic reticulum (ER) α-glucosidases I and II and thus are sensitive to glucosidase inhibitors. We and others have reported imino sugars, such as deoxynojirimycin (DNJ) and its derivatives as glucosidase inhibitors and broad spectrum antiviral agents. In our efforts to improve the antiviral efficacy of imino sugars, we discovered that introduction of an oxygen atom into the alkyl group either as a tertiary alcohol or as an ether could lead to a potent inhibitor, such as 5-(1-hydroxycyclohexyl)-N-pentyl-DNJ (OSL-95II) or IHVR-11029, demonstrated significant improved potency against bovine viral diarrhea virus (BVDV) than the classical imino sugar, N-butyl-DNJ (NB-DNJ). Here we will report our progress in further exploring the nitrogen analogs in the alkyl group by using parallel synthesis and medicinal chemistry principles. A novel class of N-alkyldeoxynojirimycins(NADNJs) possessing tertiary amide/carbamate moiety was discovered and following optimization resulted in promising compounds with improved chemical and metabolic stability, which displayed submicromolar EC_{50}s against BVDV, Dengue, and Tacaribe viruses. Moreover, the two lead imino sugars significantly reduced the mortality of the two most dreadful hemorrhagic fever viruses, Marburg virus and Ebola virus, in mice.

141 Mutations Associated with ST-246 Resistance Are Not Found as Inter-strain Polymorphisms Among a Total of 164 Orthopoxviruses

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ST-246 (Arestvyr™) is currently in clinical development for the treatment of *Orthopoxvirus* (OPV)-related infections, including smallpox. The molecule inhibits the wrapping of infectious virions by interfering with the virally-encoded F13L protein. The appearance of resistance to drugs is a major concern in clinic, and yet little has been reported on ST-246. Here, we studied how ST-246-resistance ($ST\text{-}246^R$) emerged in three OPV species grown *in vitro* under ST-246 selective pressure. To this end, vaccinia (VACV), cowpox (CPXV) and camelpox (CMLV) viruses with a reduced sensitivity to ST-246 were isolated. Each of them depicted variable levels of resistance to the drug, ranging from 10- to more than 9,000-fold increase in ST-246 EC_{50} values, as confirmed by antiviral assays. The growth of $ST\text{-}246^R$ viruses was similar to that of their respective wild-type counterparts. Four amino acid changes were identified in the F13L protein, and production of recombinant viruses demonstrated their role(s) in conferring resistance to ST-246, whereas cidofovir and CMX001 conserved their antiviral potency. To further examine whether these mutations were naturally occurring polymorphisms in the *F13L* gene of OPVs, and thus might potentially

impact ST-246 efficacy, we sequenced the *F13L* gene of 65 CPXV clinical isolates and performed alignment studies together with 99 NCBI available F13L OPV sequences. While this allowed us to identify 21 amino acid variations in 77 CPXVs, and 7 and 2 in, respectively, 49 variola and 10 monkeypox viruses, none of these substitutions mapped to hot spot regions linked with ST-246^R. In conclusion, we identified three regions in the F13L protein where ST-246^R is likely to develop and showed that they are not naturally present in a large collection of OPVs. Further modeling studies will help us to better understand the mode of action of ST-246.

142 ABCE1 is a Host Factor Involved in Capsid Assembly for Multiple Viral Families

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It has previously been shown that the host protein ABCE1 (ATP binding cassette E1), also known as ribonuclease L inhibitor or HP68, is critical in post-translational events in immature HIV capsid assembly, demonstrated first in a cell-free system and then in cells, and more recently for rabies virus in a cell-free system. We have extended the study of ABCE1 involvement during the capsid assembly process to members of other viral families, including members of the *Arenaviridae* (Lassa virus), *Filoviridae* (Marburg virus), *Orthomyxoviridae* (Influenza virus), and *Hepadnaviridae* (Hepatitis B virus). Viral capsid proteins were expressed in a cell free extract, and the products separated on velocity sedimentation gradients followed by co-immunoprecipitation across the gradient fractions with an affinity purified rabbit polyclonal antibody against the C terminus of human ABCE1 or with irrelevant affinity purified antibody control . Our findings presented here support a model in which capsid proteins of different viruses assemble into immature capsids via a stepwise pathway of assembly intermediates which share in common utilization of the host factor ABCE1 at certain stages of the assembly process. Furthermore, our data, in agreement with a previous study, suggests the involvement of selective ABCE1 subsets in assembly of capsids from different viral families. Antibodies currently being generated to distinct domains of ABCE1 may elucidate the basis for these features.

143 Castalagin as an Antiviral with Anti-herpesvirus Potential

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Our previous studies showed that castalagin, a nonahydroxyterphenoyl-bearing C-glycosidic ellagitannin, manifests a significant antiviral activity against HSV-1 and HSV-2 strains both sensitive and resistant to ACV Moreover, the combination effect of ellagitannins with acyclovir was markedly synergistic, especially the effect in the combination towards the replication of HSV-1 sensitive strain [Vilhelmova et al., Antiviral Res. 89, 2011, 174-181 Antiviral Res. 90/2, 2011, N144, A64-A65]. Here we present the results on our study on the effects of castalagin (i) on the viral adsorption of HSV-1 (*Victoria*, an ACV sensitive strain) in MDBK cells, and (ii) on the extracellular HSV virions – as an initial step of a clearing up the mode of anti-herpesvirus activity of this compound. Castalagin at the concentration of 10 μM (MTC) showed a marked effect on virus adsorption: Δlog 1.7 on the 30 min, 2.2 logs on the 45 min and 3.2 logs on the 60th min. A well expressed inhibitory effect of 2 logs was registered by 1 μM at the 60 min. At a concentration of 0.1 μM effect the effect was clearly weaker (Δlog 1) and at a concentration of 0.01 μM a suppression of virus adsorption was not observed. The compound showed a marked direct inactivating effect on extracellular HSV-1, which is markedly temperature-dependent. The effect was significantly higher at 37^{o}C: castalagin 10 μM decreased the infectious virus by Δlog 1.6 at an exposure time of 30 min, 1.8 logs at 60 min, 2 logs at 90 min, and 2.2 at the 120 min. This activity was strongly dose-dependent and exposure time-dependent: 30 min at the castalagin concentrations of 10μM and 1 μM, 60 min at 0.1 μM and 0.01 μM.

144 Cell-Free Protein Synthesis-Based Screens for Host-Targeted Antiviral Drug Discovery

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There is a growing appreciation that the most effective ways of targeting viruses may be to block their access to host proteins essential to the virus lifecycle. But identification of antiviral compounds has generally required a non-toxic starting point since, in cells, it is difficult to score anti-viral efficacy in the context of serious toxic liabilities. It is likely that compounds that target host proteins will, at the outset, be highly toxic, but that with structure-activity relationship (SAR) optimization, it may be possible to improve their profile. Thus the forementioned limitation of starting with a toxic compound poses a challenge to host-targeted drug discovery. To overcome this limitation, we have used cell-free protein synthesis (CFPS) to develop small molecule screens that carry out the central biological process of protein synthesis in the context of the complexity approximating the cytoplasm, but without the full toxicological liabilities associated with living cell systems. In principle, it should be possible to start with compounds far more toxic than are generally viable for cell-based screens and improve them through SAR. Here we will show two striking correlations that have emerged from our studies: 1) between efficacy of compounds discovered in by CFPS and their efficacy against infectious virus in cell culture; and 2) the ability of SAR optimization to take weak and toxic compounds, identify more potent analogs and then, through further analog assessment, to diminish toxicity. We propose these approaches as general methods of host-targeted antiviral drug discovery.

145 A New and Five Known Antiviral Diterpenoids from the Leaf of *Pinus densiflora* Against Human Papillomavirus *in vitro*

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The traditional Chinese medicine, *Pinus densiflora* S. et. Z, has been used for the treatment of hemorrhage, diarrhea and various inflammations. Previous studies on *P. densiflora*, have investigated various biological activities including anticancer, antimicrobial, antioxidant, anti-diabetic, anti-obesty and actylchlinesterase inhibitory effects. Gental human papillomavirus (HPV) infection is the most common sexually transmiited infection, and virtually most of cervical cancers are attributable to HPVs infection. In continuation of our search for anti-HPV compound from natural products, we have found that the methanol extract of pine leaf has anti-viral activity against HPV16PVs, and recently, we reported anti-viral diterpenoids and a sesquiterpene from the dichloromethane fraction of pine leaf, which showed comparatively higher activity than the other fractions. Further separation of the active fraction has yielded a new and five known additional diterpenoids. The structure of a new diterpenoid was determined as (13S)-15-hydroxylabd-8(17)-en-18-oic acid (1) by spectroscopic analysis. Five known compounds were identified as 7α-hydroxycallitirisic acid (2), 13-oxo-15,16-dinorlabda-8(17),11E-dien-19-oic acid (3), 13-hydroxy-8,11,13-podocarpatrien-18-oic acid (4), ent-labd-8(17)-ene-15,18-dioic acid (5), 7-oxo-15-hydroxydehydroabietic acid (6) by comparison of their sepectral data with literature values. Anti-viral activities for the dichloromethane fraction and isolated compounds were evaluated using bio-luminescence(SEAP) assay on HPV16PVs infected 293TT cells. The anti-viral effect of dichloromethane fraction increased 10% to 94% depending on their treat times. Compounds 16 exhibited 80~100% reduction of HPV16PVs at concentration 100μg/mL.

146 Comparison of Chikungunya Virus Isolates in Mice with Regard to Pathogenesis and Sensitivity to Effective Antiviral Treatment

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Infection with Chikungunya virus (CHIKV) generally results in a debilitating arthralgia. Mortality rates are low, but the majority of infections are symptomatic. Recent outbreaks were associated with changes in the envelope protein of the virus that enhanced transmission by Aedes albopictus and resulted in increased rates of symptomatic infection and greater disease severity. Since the emergence of this viral variant in 2006, millions of CHIKV cases have been reported, underscoring the need for a suitable model to identify potential therapies. We compared the pathogenesis of an east African strain (S27) with the epidemic strain LR2006-OPYI (LR06) in DBA/1J mice infected via footpad/hock injection. Animals inoculated with LR06 had an average of 78% increase in joint swelling at the site of inoculation as compared with a 43% increase in animals inoculated with S27 at an equivalent infectious challenge (50% cell culture infectious dose). In addition, swelling was observed on day 3 after virus challenge (64% increase) in animals inoculated with LR06, but was absent at the same time point after S27 inoculation. These observations demonstrate a difference in pathogenesis between these two CHIKV isolates. Virus titer increase in various tissues was compared for the two strains. Cytokine and chemokine levels in the hind leg at the site of footpad swelling were also characterized. Treatment of mice 24 h prior to virus challenge with 10^7 pfu of mDEF201, an adenovirus-vectored interferon, significantly reduced swelling in animals challenged with either strain of CHIKV, demonstrating the effectiveness of this agent in treating a severe CHIKV infection. Infection of DBA/1J mice with LR06 CHIKV appears to be a more robust model of disease as compared with S27 infection and also shows utility in antiviral studies. The increased pathogenesis associated with the LR06 further provides a relevant model for the discovery of therapies to reduce the burden of CHIKV infection. [Supported by HHSN272201000039I Task Order A21 from the Virology Branch, NIAID, NIH]

147 Isoflavone Agonists of IRF3-dependent Signaling Elicit Potent Antiviral Activity Against Diverse Respiratory Viruses

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There is a strong demand for broad-spectrum antivirals that retain potency and efficacy in the face of rapidly evolving viral genomes. Activating cellular innate antiviral effectors is an attractive strategy in development of a pan-tropic antiviral. To this end we have refined an isoflavone small molecule agonist of the RIG-I-like receptor (RLR) pathway to elicit potent antiviral activity against a number of diverse RNA viruses. The parent isoflavone compound, KIN101, was identified in a screen for small molecules that activate IRF3-driven transcription. KIN101 was found to activate IRF3 nuclear translocation to drive the specific upregulation of approximately 65 gene targets involved in innate immunity, and exhibits modest *in vitro* antiviral activity against Hepatitis C Virus and Influenza. Refinement of structural components of KIN101, through structure activity relationship (SAR), resulted in an analog, KIN269, which exhibits nanomolar *in vitro* antiviral activity across multiple classes of viruses including those from orthomyxovirus and paramyxovirus families. These families represent both nuclear and cytoplasmic-replicating respiratory viruses, highlighting the capability to restrict diverse virus lifecycles. Importantly, KIN269 shows significant therapeutic benefit in an *in vivo* Influenza A mouse model. Intranasal administration of even a single prophylactic dose significantly decreased both morbidity and mortality from Influenza A in a lethal challenge. Mice receiving prophylactic treatment of KIN269 who did develop clinical signs of disease displayed a delay in the onset of symptoms, overall decreased clinical symptoms and an increased mean time to death compared with vehicle treated mice. In summary, we have developed a non-direct-acting antiviral that potentiates a cell autonomous effector response that is active against diverse respiratory viruses, is less likely to elicit the emergence of resistant viral variants, and has potential to be used as a therapeutic for respiratory infections of undiagnosed etiology.

148 Selective Antiviral Activity of a Novel Compound Class Developed *in silico* Based on the Structure of the E Protein of Dengue Virus

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The dengue virus (DENV) envelop (E) protein contains a hydrophobic pocket, called the ß-OG pocket, located between domains I and II. Flexibility of the E protein at the level of this cavity is required during fusion of the virus particle with the host cell membrane. We previously reported that the ß-OG pocket of the DENV E protein might be an excellent target for the development of small-molecule inhibitors of DENV entry (Kaptein *et al*, Antimicrob. Agents and Chemother. 54, 2010). Based on the crystal structure of the E protein, a panel of forty-four molecules was evaluated in a CPE-based reduction assay for DENV-2. Compound 23 (belonging to the class of 2-[(2E)-2-benzylidene/-(1-phenylethylidene)-hydrazinyl]-4-phenyl-1,3-thiazoles) was identified as a selective inhibitor of *in vitro* DENV-2 replication (EC_{50} = 5.8 ± 1.2 µM CC_{50} = 145 ± 12 µM), in particular when virus and molecule were pre-incubated for 2h at 37 °C. The antiviral activity was confirmed in a virus yield reduction assay using DENV-2 (EC_{50} = 0.9 ± 0.3 µM). Moreover, compound 23 inhibited the replication of the other three DENV serotypes (EC_{50} values ranging from 1.2 - 3.8 µM) as well as YFV-17D (EC_{50} = 0.9 ± 0.2 µM). Compound 23 was shown to be devoid of virucidal activity, indicating that the compound does not disrupt the integrity of the virus particles. Given the fact that pre-incubation of virus with the compound is required to produce an antiviral effect, it can be concluded that compound 23 exerts it antiviral activity at an early stage of the virus life cycle (entry/uptake). Studies are ongoing to explore how exactly the compound targets the DENV E protein.

149 A High-throughput Assay for Assessment of Antiviral Activity Against Human Norovirus Proteases

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Human Noroviruses (NoV) are a group of related, non-enveloped, single-stranded, positive sense, RNA viruses that are responsible for at least 50% of all epidemic gastroenteritis outbreaks worldwide. In the US, NoV infect 21 million annually with 70,000 hospitalizations, are responsible for 58% of foodborne illness, and are the most frequent hospital-acquired infection, accounting for 65% of all hospital unit closures. Worldwide, NoV infections annually cause over 1.1 million hospitalizations and more than 220,000 deaths among children in developing countries. Currently, there are no antivirals directed at NoV available and no clinical trials are in progress. Drug discovery in this field has been primarily hampered by a lack of reagents suitable for efficient antiviral investigation. We developed a sensitive, fluorescence-based, *in vitro* assay for human NoV proteases that operates at physiologic pH. HEPES was superior to Tris-HCl or sodium phosphate, and buffer concentrations above 10mM were notably less efficient. Cations (Na^+, K^+, Mg^{+2}, Mn^{+2}) inhibited protease activity by affecting the rate of reaction (k_{cat}), but did not affect affinity for the peptide substrate (K_m). Differences in efficiency due to pH, and buffer composition or concentration also affected k_{cat} but not K_m. We optimized and validated the assay for HTS with final conditions of: 10mM HEPES (pH 7.6), 0.1% CHAPS, 5mM DTT, 4% DMSO, 0.25µM NoV protease, 25µM substrate peptide, 5µL/well in 1536-well plates. The average Z´-value for the validation was 0.62, and the average %CV was 6.60%. A pilot screen using a small diversity set of 20K compounds was performed. The median Z´-value for the 16 assay plates used in the screen was 0.80 (range, 0.71 to 0.86), and the median %CV values for the low and high signal controls were 2.55% and 2.88%, respectively. The availability of a robust, reproducible, and high quality HTS assay now enables routine progress in antiviral discovery against this significant public health problem.

150 New Prodrugs of Acyclic Nucleoside Analogues and Phosphonomethyl Derivatives

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Development of prodrugs of 3-hydroxy-2-(phosphonomethoxy)propyl (HPMP) derivatives is rather difficult due to the presence of a free OH group leading preferentially to formation of free cyclic phosphonates. In our group we developed methodologies for easy, one step transformation of parent HPMP derivatives to appropriate prodrugs – POM esters and amidates (Fig.1). This methodology has been already applied to (*S*)-HPMPC (cidofovir) and its 5-azacytosine analog, (S)-HPMP-5-azaC but it was found generally usable for all HPMP derivatives.Transformation of cidofovir or HPMP-5-azaC to amidates proceeded in both cases under formation of phosphoramidates of the cyclic form and single diastereoisomers can be separated by crystallization. An alternative strategy for synthesis of prodrugs are enzymatic reactions, e.g. glycosylations, using transglycosylation activity of β-galactosidase from *E. coli* to form appropriate β-galactosides. The method can be applied to ANP prodrugs functionalized by hydroxyl group(s) in their ester moiety. The final aim of this approach is preparation of tissue specific prodrugs. Another enzymatic method is preparation of fatty acid esters using transesterification activity of lipases. Thus far, the method has been successfully used to synthetize esters derived from antiherpetic agent DHPA, 9-(2,3-dihydroxypropyl) adenine.

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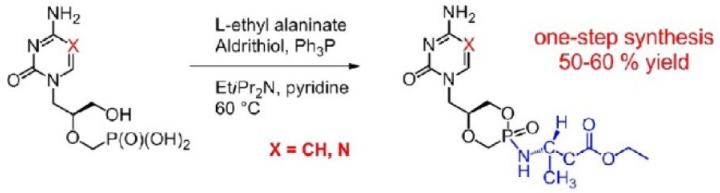

151 Novel Tyrosine N-alkyl Amide Prodrugs of (S)-HPMPC and (S)-HPMPA: Preliminary SAR Studies and *in vitro* Antiviral Activities Against HCMV, Cowpox and Vaccinia Viruses

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(*S*)-HPMPC (cidofovir, Vistide®) and its adenine analogue, (*S*)-HPMPA are acyclic nucleoside phosphonates (ANPs) that are potent against a broad spectrum of DNA viruses, including human cytomegalovirus (HCMV), cowpox and vaccinia. In order to ameliorate poor oral bioavailability and low cellular uptake of these ANPs, we are developing an amino acid-based prodrug approach (*Mol. Pharm.,* **2013**, *10*, 445). In a preliminary structure-activity relationship (SAR) study focused on derivatization of the carboxyl group in the amino acid promoiety, we synthesized a series of tyrosine *N*-alkyl amide conjugates of (*S*)-HPMPC and (*S*)-HPMPA, incorporating alkyl chains varying in length from 4 to 18 C-atoms. *In vitro* antiviral activities (IC_{50} values) of the prodrugs obtained against HCMV, cowpox and vaccinia viruses showed 1-4 logs greater activity than the parent ANPs (0.05-4 μM). The potencies of cyclic and acyclic phosphonate prodrugs, incorporating alkyl chains of the same length were comparable despite 3-4 logs difference in their calculated log *D* values. The oral bioavailability of the tyrosine *N*hexadecyl amide prodrug of (*S*)-HPMPC was 46% and 33% in fed and fasted mice, respectively.

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152 Molecular Mechanism of Action of LPCRW_0005, a Benzonitrile Derivate, on the Replication of Human Rhinovirus 14

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In a large-scale, virus-cell-based antiviral screening effort against human rhinovirus 14 (hRV14), 4-(1-hydroxy-2-(4,5-dimethoxy-2-nitrophenyl)ethyl)benzonitrile (LPCRW_0005) was identified as a selective (EC_{50} of 2 ± 1μM with 100% inhibition of virus-induced CPE at concentrations of 13μM CC_{50} of 178μM (selectivity index of 89) inhibitor of virus replication. A total of 97 structural analogues were synthesized and a structure-activity-relationship was established. Evaluation of the selective antiviral effect of the most potent compounds on fifteen different hRV serotypes, including major and minor group serotypes, revealed a specificity of the compounds for hRV14. A time-of-drug-addition study demonstrated that LPCRW_0005 interferes with the earliest stages of virus replication. Drug-resistant virus isolates were selected for LPCRW_0005 and either an A150V or A150T amino acid mutation was observed in the VP1 capsid protein. All of the selected putative compound-resistant virus isolates showed >10 or >30-fold resistance to the antiviral effect of LPCRW_0005. Reverse engineering of these amino acid mutations into an infectious clone of hRV14 is ongoing and will provide insight in the role of the different residues in the resistant phenotype. Cross-resistance with the capsid binder pleconaril was observed and, vice versa, the replication of pleconaril-resistant virus isolates was not inhibited by LPCRW_0005. Molecular modeling of LPCRW_0005 in the structure of the wild-type and mutant VP1 capsid protein of hRV14 suggests that steric hindrance, caused by the A150V or the A150T mutation, prevents interaction of the molecule with its target and thus explains the loss of antiviral activity.

153 Molecular Dissection of the Timing and Mechanism of Action of a Small Molecule with Robust Activity Against Host Factors that Participate in Rabies Virus Capsid Assembly

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We have adapted a classical cell biological technique, cell-free protein synthesis (CFPS), into a novel approach to antiviral drug discovery. We developed a CFPS system to reconstitute early events in rabies virus (RABV) capsid protein biogenesis, which allowed us to detect previously unappreciated host-virus protein-protein interactions. On the hypothesis that these protein-protein interactions could represent a druggable target, we utilized the RABV CFPS system as a whole-pathway screen to identify small molecules interfering with the putative host-catalyzed RABV capsid assembly pathway. Hits from this screen were improved through structure-activity relationship optimization and corroborated by robust activity against infectious RABV in cell culture. Previously, we coupled active anti-RABV compound PAV-866 to a resin to use as a ligand for affinity chromatography, to retroactively identify the target. We showed the target to be a host multi-protein complex. Here we present data suggesting that the PAV-866 binding site changes its accessibility from one intermediate to another. Moreover, PAV-866 is likely not directed to the substrate binding site, but rather to an allosteric site of the target, because binding to PAV-866 does not displace radiolabelled RABV nucleoprotein and phosphoprotein substrates.

154 Proteolysis Inhibitor Aminocaproic Acid Shows Both Antiviral and Antibacterial Activity

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We have shown that the proteolysis inhibitor (PI) – Aminocaproic acid (ACA) demonstrate the antiviral activities towards some influenza viruses. ACA prevents the enhancement of proteolysis during cell membranes-virions interaction, decreases penetration of virions into sensitive cells. It brings down proteolytic cleavage of HA precursor to HA-1 and HA-2 and reduces the virus harvest. High levels of ACA anti-influenza efficacy were shown on subtypes H1N1; H2N2; H3N2 and type B human influenza viruses. ACA promotes the intensification of specific antibodies production and cell immunity, prevents vessels' permeability and hemorrhagic phenomena and decreases the destruction of bronchi's epithelium. On the basis of our experiments and clinical trials Ukrainian Ministry of Health had allowed using ACA as antiviral for prevention and treatment influenza and other ARVI in children and adults. Also we have studied action of ACA on H5N3 and H7N3 avian influenza viruses. H5N3 is more sensitive to ACA than H7N3. It is known that *Staphylococcus aureus* are often the cause of bacterial complications of the influenza. We have established that ACA inhibits the reproduction of strains of *S.aureus* that have different sensitivity to antibiotics (ABs). The combined use of ABs with ACA increases their antibacterial efficacy against pathogens. According our hypothesis, the PI blocks or decrease the activity of the bacterial enzymes directed to the AB destruction. PIs could defend the ABs from the destructive action of bacterial enzymes and enhance the sensitivity of the microorganisms to ABs. Thus, the combined use of PIs with ABs could cause the changes of microorganism's metabolism and in such a way to decrease the effective doses of ABs that diminishes the toxicity of the treatment. CONCLUSIONS: ACA is effective antiviral against influenza viruses. The use of ACA in treatment of influenza is also prevention of bacterial complications. Use ACA for treatment of bacterial complications of the influenza allow to increase the sensitivity of the microorganisms to the ABs and decrease their doses. ACKNOWLEDGEMENT: This research is partially supported by STCU Grant P407/KCP T2-269.

155 The Benzamide-based Compound K22 Exhibits Anti-coronavirus 229E Potency by Targeting the Activity of Viral Nonstructural Protein 6

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Coronaviruses (CoV) are enveloped, positive stranded RNA viruses that can infect human airways and cause respiratory symptoms ranging from common cold to severe respiratory illness. In spite of the fact that some CoV can be regarded as potential pandemic-causing viruses, no specific antiviral drug is currently available for treatment. A feature of CoV infections of cells is that these viruses modify intracellular membranes to form a reticulovesicular network (RVN) required for the virus replication in cytoplasm. These changes are believed to be generated by a number of viral proteins including the hexaspanin nonstructural protein 6 (nsp6). In this study we have identified a potent benzamide-based inhibitor of CoV 229E infectivity, referred to as K22, which appears to target the biological activity of viral nsp6. K22 displayed an antiviral IC_{50} value of 0.7 μM while showing no toxicity for MRC-5 cells at 20 μM (solubility limit). Results of the time-of-addition assay revealed that the drug exhibited antiviral activity when added up to 6 h after infection of cells manifested in qPCR analysis as near-complete inhibition of viral RNA synthesis. In addition, we observed that K22 inhibited formation of the CoV-induced clusters of double membrane vesicles of the RVN. Moreover, two independently generated viral variants resistant to K22 exhibited amino acid substitutions of H121L and M159V, respectively, both occurring in viral nsp6. The production of recombinant viral variants displaying either the H121L or M159V, or both of these amino acid substitutions confirmed that the nsp6 activity is a target for K22. It is likely that the role nsp6 plays in the assembly of CoV RVN represents a novel and attractive target for antiviral intervention with candidate drugs such as K22. Altogether, the benzamide-based K22 is an anti-CoV 229E lead compound with a novel mechanism of action which, in addition to its antiviral activity, may help to clarify some of the complex features of CoV replication.

156 Interactions of Acyclic Nucleoside Phosphonates with Selected Renal SLC and ABC Transporters

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Acyclic nucleoside phosphonates (ANPs) are nucleotide analogues well-known for their promising antiviral and anti-proliferative properties. As they are negatively charged under physiological conditions, clinically important ANPs may cause renal injury via accumulation through organic anion transporters (OATs). The goal of the study was to study interactions of several structurally related and biologically potent ANPs with selected human SLC transporters (hOAT1, hOCT2, hCNT2, and hCNT3) and ABC transporters (ABCB1 and ABCG2) using appropriate cell models and compare the results with the clinically approved antivirals adefovir and tenofovir. Among the tested influx transporters, the majority of the studied ANPs showed their potency to interact with hOAT1, while the other tested renal influx transporters hOCT2, hCNT2, and hCNT3 seemed to be insignificant for the uptake of the tested ANPs in proximal tubules. The double prodrug of PMEG, the compound GS-9191, displayed high affinity for hOAT1, comparable with that of adefovir and tenofovir. Other tested ANPs expressed substantially lower affinity for hOAT1 than GS-9191. Furthermore, among the ANPs tested, only GS-9191 exhibited a significant interaction potential with efflux transporters ABCB1 and ABCG2. The tested compounds caused significant decrease in cell viability in the cells transiently transfected with hOAT1 and the most cytotoxic compound was the lipophilic analogue GS-9191. In most cases, the cytotoxicity of the ANPs tested closely corresponded to their potential to interact with hOAT1. In conclusion, it was demonstrated that hOAT1 has a considerable impact on the pharmacokinetics of the ANPs tested and may play an active role in the mechanism of nephrotoxicity of ANPs. ACKNOWLEDGEMENTS This work was supported by Charles University in Prague (Project SVV: 265003), by the Institute of Organic Chemistry and Biochemistry AS CR in Prague (Project RVO: 61388963), grant GAUK No. 360811/FaF/C-LEK, and grant IGA MZ No. NT12398-4/2011.

157 *In vitro* Study of the Effect of Nitazoxanide on the Replication of Dengue Virus and Yellow Fever Virus

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Nitazoxanide (NTZ) has being used to fight infection against a wide number of bacteria, protozoa and viruses. In this scenario, the inhibition of the replication of the Hepatitis C virus by this molecule raised the question whether NTZ could inhibit the replication of Dengue viruses, since both viruses belong to the same family (*Flaviviridae*), although clinically distinct diseases. Dengue virus is one of the most important problem of public health in over 100 Countries, and according to the WHO about 2,5 billion individuals are in risk of the infection, infecting about 50 million individuals each year. Although great effort is directed to this virus, no licensed vaccine exists. This study evaluates the effect of Tizoxanide (TZX), the primary metabolite of NTZ in the replication of Dengue virus *in vitro*. Since NTZ is already being used in human population for the treatment of other adversities, there is a great possibility that NTZ can be used to reduce morbidity and mortality of Dengue around the world. We also tested the effect of TZX in the replication of the Yellow Fever Virus, another Flavivirus. Although there is an effective vaccine against this virus, there are important issues such as the high mortality rate of infected individuals and also the fact that some vaccinated individuals may acquire Yellow fever. Vero cells were infected with either Dengue virus – serotype 2 (New Guinea strain) or the vaccine strain 17DD of Yellow Fever virus and then treated with non-toxic concentrations of TZX as determined by neutral red assay. Our results indicated that 1,0 uM of TZX inhibited the replication of Dengue virus by 91,6%, with a Selectivety Index (value of CC_{50}/IC_{50}) of 4.8. For Yellow Fever, even smaller concentrations of TZX (0.25uM) inhibited more than 90% of virus replication, with a Selectivety Index of 35. In this study we show for the first time that Nitazoxanide should be evaluated as a potential antiviral against Dengue and Yellow Fever infections.

158 Obscurum per Obscurius: Computer-aided Design of Novel Antivirals Using Simplex Approach

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Viruses are one of the important causes of human diseases, such as hepatitis, AIDS, or influenza. Depending on the strain and the person's state of health, they can infect almost any type of body tissue, from the brain to the skin and hit almost everyone. The burden of only influenza in the USA is currently estimated to be 25–50 million cases per year, leading to 150 thousand hospitalizations and up to 30-50 thousand deaths. Here we report on the application of the Simplex representation of molecular structure (SiRMS) for QSAR modeling in antiviral research. After introducing the field of cheminformatics, we present the SiRMS approach: SiRMS represents every molecule as a system of different simplexes (tetratomic fragments with fixed composition, structure, chirality, and symmetry). The main advantages of SiRMS are: *(i)* consideration of the different physical-chemical properties of atoms, *(ii)* high adequacy and good interpretability of developed models, and *(iii)* clear workflow for computer-aided molecular design. The SiRMS approach is implemented within the "HiT QSAR" software, which is available from the authors by request. The reliability of developed QSAR models as predictive virtual screening tools and their ability to serve as the guide for targeted drug design was validated by synthetic and biological experiments. We discuss several applications of SiRMS in antiviral research and future directions of extending Simplex approach for QSAR analysis of mixtures. Keywords: *computer-aided molecular design, SiRMS, HiT QSAR.*

159 Conformational Flexibility of the Dengue Virus RNA-Dependent RNA Polymerase Revealed by a Complex with an Inhibitor

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Dengue is a mosquito-borne virus that is a major threat to human health in tropical and subtropical regions. We report a highly reproducible method to crystallise the RNA-dependent RNA polymerase (RdRp) domain of dengue virus serotype-3 (DENV-3), allowing structure refinement to 1.79 Å resolution and revealing amino acids not seen previously. We also present a DENV-3 polymerase/inhibitor co-crystal structure at 2.1 Å resolution. The inhibitor binds to the RdRp as a dimer and causes conformational changes in the protein. The improved crystallisation protocol and new structural information will assist structure-based drug discovery.

160 Anti-poliovirus Compounds from Medicinal Plants Selected from Tthe Nigerian Ethno-medicine

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Medicinal plant have been a constant source of safe and effective drug, believed to be capable of providing local community with familiar, acceptable, safe and effective source of anti-poliovirus drug and also capable of providing the scientific world with more weapons to fight on, even after eradication. Fourteen medicinal plant samples, obtained for a previous ethnobotanical survey, were extracted by maceration into absolute methanol at room temperature and subjected to antiviral assay using *In vitro* MTT neutralization method, rabbit antiserum was used as positive control. Data obtained were analyzed statistically using GraphPad prism, Neutralization index (NI), calculated as the ratio of IC_{50} to the least concentration tested, was used to compare the activity of tested samples with the control. The most active extracts from whole plant of *Zephyranthes candida and* stem bark

of *Cassia siamea* were subjected to bioassay-guided fractionation, repeated column and preparative thin layer chromatography led to isolation of active compounds. Chemical structures of compounds were elucidated using spectroscopic techniques. Crude extracts from *Z. candida and C. siamea* were the most active of the extracts with IC_{50} of 0.018 µg/mL and 0.12 µg/mL respectively. Lupeol (IC_{50}, 0.014 µg/mL) and lycorine (IC_{50}, 0.0058 µg/mL) were the most active compound isolated from the medicinal plants. Two other active compounds, 7-hydroxy-3›, 4›-methylenedioxyflavan and trisphaeridine with good selective index (SI) were obtained from *Z. candida.* The studies revealed that in the context of antiviral drug development, methanol extracts from the two active plant may play significant role by providing chemical entities that may be lead for development of antipoliovirus agents.

161 Human Cytomegalovirus Resistance to Deoxyribosylindole Nucleosides Maps to a Point Mutation in the Terminase Subunit Encoded Gene UL89

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Human cytomegalovirus (HCMV) infection can result in severe disease including retinitis and encephalopathy in immunocompromised patients and mental retardation, vision and/or hearing loss in immunologically immature patients. Current FDA-approved therapies for managing HCMV infections include ganciclovir (GCV), cidofovir, and foscarnet, but these can result in serious adverse effects including hematological abnormalities or nephrotoxicity. The deoxyribosylindole nucleosides constitute a new class of compounds that demonstrate 20-fold greater activity (IC_{50} = 0.34 uM) compared to GCV (IC_{50} = 7.4 uM) without any observed increase in cytotoxicity. Previous studies have demonstrated that HCMV resistant to the chemically related benzimidazole ribonucleosides is also resistant to indole nucleosides. The benzimidazoles act late in the viral replication cycle by inhibiting the viral terminase but are not viable clinical candidates due to poor pharmacokinetic profiles *in vivo*. The HCMV terminase, encoded by genes *UL56* and *UL89*, is an enzyme that cleaves high-molecular-weight DNA concatemers into genome length units and is necessary for viral genome processing and packaging, thus making it an excellent target for antiviral chemotherapy. We, therefore, hypothesize that the indole nucleosides target the HCMV terminase. To test this hypothesis, an indole-resistant HCMV was isolated, its genome sequenced, and a G766C base pair mutation in the exon 1 of *UL89* was identified. This mutation resulted in an E256Q amino acid change, which is unique and distinct from the mutations previously discovered that confer benzimidazole resistance (D344E and A355T). We surmise that this mutation is responsible for HCMV resistance to the indole nucleosides and further studies are ongoing to confirm this hypothesis.

162 Cellular Factors Involved in Dengue-3 Virus Entry into the Host Cell

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Dengue virus (DENV) is an arthropod-borne member of *Flaviviridae* that can cause in the man a feverish and self-limited disease, the dengue fever, or more severe and potentially lethal forms of dengue hemorrhagic fever/ dengue shock syndrome. Currently there are no available vaccines or antiviral therapies. Due to the co-circulation of the four DENV serotypes (DENV-1 to 4) in the same regions and the chance of heterologous antibody-dependet enhancement of the infection, any therapeutic or preventive development must be protective against all serotypes. Otherwise, the knowledge of the viral multiplication cycle is critical for the finding of potential antiviral targets. In this field, the initial steps leading to DENV entry into the host cell, at present poorly understood, represent an interesting antiviral strategy. Here, the mode of entry of DENV-3 to mammalian cells was studied by analyzing the effect of pharmacological inhibitors of different endocytic pathways on virus infectivity and antigen expression

in monkey Vero and human A549 cells. DENV-3 entry into both cells was strongly inhibited by treatment with ammonium chloride or concanamycin A, two inhibitors of endocytosis which raise the endosomal pH. The presence of inhibitors of clathrin-mediated endocytosis, chlorpromazine or dansylcadaverine, did not affect virus entry whereas dynasore reduced DENV-3 internalization, indicating the involvement of dynamin entry. A slight effect on virus infection was produced by nystatin and methyl-β-cyclodextrin, two cholesterol-binding agents. Then, our results provide evidence that DENV-3 enters into Vero and A549 cells by a low pH-mediated, clathrin-independent endocytosis route, dependent on dynamin. Furthermore, the comparison with our previous studies with DENV-1 and DENV-2 confirms that diverse viral and celullar factors affect DENV entry and should be considered for evaluation of safe antiviral agents reactive against all DENV serotypes.

163 *In vitro* Antiviral Activity of β-carbolines Against RNA Viruses

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The β-carboline skeleton is present in many natural and synthetic products associated with biological activities. The natural harmol, harmine, harmane, norharmane and their synthetic 9-methyl derivatives were evaluated for their antiviral activity *in vitro* against enveloped viruses with RNA genome: two human pathogens, the hemorrhagic fever viruses dengue type 2 (DENV-2) and Junin virus (JUNV), and vesicular stomatitis virus (VSV), which has veterinary importance. First, Vero cells were incubated with different concentrations of the compounds and after 48 h of treatment cell viability was determined by the MTS method and the 50% cytotoxic concentration (CC_{50}) was determined. Harmine was the most cytotoxic compound suggesting that the C-7 methoxy group adversely affect cell viability. All N-methylated derivatives display higher CC_{50} values than natural compounds indicating that methylation of the pyrrole ring caused a marked reduction in the cytotoxicity. The antiviral activity was determined by a virus yield inhibition assay. To this end, cells infected with VSV, DENV or JUNV were treated with non-cytotoxic concentrations of each compound for 48 h and virus yields were quantified by plaque assay. The 50% effective concentration (EC_{50}) was determined and the selectivity index (SI) was calculated as the ratio CC_{50}/EC_{50}. Harmane and N-methyl-harmane were active against VSV with SI values of 16 and 30.6, respectively. Harmol and N-methyl-harmine were active against DENV-2 with SI values of 56.2 and 61.2, whereas N-methyl-norharmane was the most active compound against JUNV (SI=15). The active compounds also achieved a clear reduction in viral antigen expression detected by indirect immunofluorescence assays. Compounds did not show direct inactivating activity, with the exception of N-methyl-harmine which exhibited a weak virucidal effect against DENV-2. Therefore, natural and synthetic β-carbolines are able to inhibit the replication of different RNA viruses, being DENV-2 the most susceptible to the action of this kind of compounds.

164 Synergistic Efficacy of Mycophenolic Acid or Mycophenolate-mofetil with Acyclovir Against Herpes Simplex Virus Type 1 in Mouse Models

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Mycophenolate mofetil (MPA-m), an immunosuppressive agent and prodrug for mycophenolic acid (MPA), is used to treat transplant recipients and others with autoimmune disorders. These drugs inhibit inosine monophosphate dehydrogenase which depletes intracellular GTP pools and inhibits viral replication. This would also increase the ratio of acyclovir (ACV)-triphosphate to the GTP competitor and we hypothesized that it should potentiate the efficacy of ACV against herpes simplex virus (HSV) *in vivo*. Mice were intranasally infected with an LD_{90} of HSV type 1, strain E-377 and then treated 24 hr post viral inoculation with MPA or MPA-m with or without acyclovir. In the first experiment, MPA was given orally once daily at 25, 7.5 or 2.5 mg/kg with or without ACV given orally twice daily at 10, 3 or 1 mg/kg. In the second experiment, MPA-m was given orally once daily at 30, 10 or 3 mg/kg with or without ACV given orally twice daily at 10, 3 or 1 mg/kg. Neither MPA nor MPA-m when given as a single

therapy had any effect on survival or mean day to death. Improved survival or increased mean day to death was observed in groups receiving combination therapies of suboptimal doses of ACV with MPA or MPA-m. Specifically, mice treated with MPA at 7.5 mg/kg with ACV at 3 mg/kg had only 53% mortality which was significantly different from vehicle treated mice ($p < 0.01$) and lower than mice treated with 3 mg/kg ACV as a single therapy. Also, mice treated with MPA-m at 10 or 3 mg/kg with ACV at 10 mg/kg had only 40 or 27% mortality respectively which was significantly different from vehicle treated mice ($p \leq 0.001$). Mice treated with MPA-m at 3 mg/kg with ACV at 1 mg/kg had only 60 % mortality which was significantly different from vehicle treated mice ($p \leq 0.05$). In conclusion, MPA and MPA-m synergistically improve survival or increase length of survival when combined with suboptimal doses of ACV. **Acknowledgments:** This work was supported by NIAID, NIH contract HHSN272201000007I.

165 Clearance of a Human Norovirus Replicon from the Host Cell by the Protease Inhibitor Rupintrivir

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Human noroviruses are a major cause of foodborne illness, accountable for 50% of all-etiologies outbreaks of acute gastroenteritis (both in developing and developed countries). These viruses affect all age groups; children, the elderly and immunocompromised are more susceptible to complications. Associations with chronic gastrointestinal problems and cases of chronic gastroenteritis caused by noroviruses are being increasingly recognized. No treatment or vaccination is available to date against norovirus infections. Rupintrivir (AG-7088) is a protease inhibitor originally developed for the treatment of human rhinovirus infections. Activity has also been demonstrated against other picornaviruses as well as against corona- and caliciviruses. Human noroviruses belong to the *Caliciviridae* family whose protease displays a chymotrypsin-like fold with a cysteine as the active-site nucleophile with similarities to the 3C protease of picornavirus. Rupintrivir has recently been reported to inhibit the replication of the Norwalk virus replicon (Kim et al., 2012). We here demonstrate that rupintrivir is also an inhibitor of the murine norovirus (MNV), a surrogate of human norovirus. Rupintrivir inhibited MNV-induced CPE formation and viral RNA synthesis with EC_{50} values of ~10 µM. The activity against the human Norwalk virus replicon is however more pronounced with an EC_{50} value of 0.3 ± 0.1 µM and an EC_{90} of 1.5 ± 0.2 µM. We demonstrate that two consecutive passages of the Norwalk replicon in the presence of 1 µM rupintrivir resulted in clearance of the replicon from the host cell. Combination of rupintrivir with either the polymerase inhibitor 2'-*C*-methylcytidine or favipiravir (T-705) resulted in a merely additive antiviral activity. Thus, the design of molecules targeting the protease of noroviruses could be a successful approach for the treatment of infections with these viruses. Supported by EU FP7 project SILVER (grant 260644).

166 Biochemical Characterization and Structural Analysis of NS2B/NS3 Protease from St. Louis Encephalitis Virus for Inhibitor Design

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The mosquito-borne St. Louis Encephalitis Virus (SLEV) is a member of the flavivirus genus. SLEV is mainly found in the United States as well as in the Caribbean, and in South American countries. Since 1964, approximately 5,000 cases have been reported in the United States. The symptoms of SLEV infection can range from a mild fever to encephalitis, coma, and paralysis. No vaccine or antiviral drug is available for the prevention or treatment of SLEV infections. All proteins of SLEV are translated in form of a polyprotein from one open reading frame and processed by host proteases and the viral NS2B/NS3 serine protease. To date, not much is known about the SLEV NS2B/NS3

protease, despite its pivotal role. The aim of our study is the structure-based design of small-molecule inhibitors targeting the SLEV NS2B/NS3 protease. To this end, we have recombinantly produced the protease, characterized its enzymatic properties, elucidated its crystal structure at 2.9 Å resolution, and tested several anti-NS2B/NS3 protease inhibitors.

167 Development of a Novel Animal Component Free Medium that Promotes the Growth of Various Animal Cell Lines for the Production of Viral Vaccines

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The production of vaccines using animal cell culture systems was one of the earliest commercial applications of *in vitro* animal cell technology. One of the important points of safety concerns relates to the composition of culture media. All classical vaccine production processes make use of animal derived substances: serum, trypsin, etc. Serum has shown several essential functions in culture: it is a source of nutrients, hormones, growth factors and protease inhibitors, etc. Nevertheless, it has some major disadvantages. It is undefined with respect to its chemical composition. It can be a source of adventitious agents and their by-products (such as bacterial endotoxins). Serum also presents a variable performance of cell growth and has a substantial cost. Therefore, the benefits of *in vitro* culture of animal cell lines in the absence of serum are widely acknowledged. We had previously developed an animal component free medium suitable for Vero cells growth under static and agitated cultures (Rourou et al. 2009a; 2009b). This medium named IPT-AFM, has a simple composition and a reasonable cost. In this work, we also describe the use of IPT-AFM to assess the growth of adherent BHK-21 cells. BHK-21 cells were first tailored to grow in suspension in serum containing medium. Then, they were adapted to IPT-AFM, by progressive reduction of the amount of serum in the culture medium. After their adaptation, BHK-21 cells grow in suspension by forming clumps. Various disaggregation components were tested in order to reduce the size of the clumps. Better results were obtained with the addition of pluronic acid associated with dextran sulfate or polyvinyl sulfate. Kinetics of cell growth were studied in agitated cultures. The kinetic parameters were comparable to those obtained with cells cultivated in serum containing medium on 2.5 g/L Cytodex 3 microcarriers. In addition, the optimization of rabies virus production (strain PV/BHK-21) in BHK-21 cells growing in suspension was investigated.

168 Antiviral Activity of Favipiravir Against Experimental Rift Valley Fever Virus Infection

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Rift Valley Fever is a zoonotic, arthropod-borne disease that affects livestock and humans. The etiologic agent, Rift Valley fever virus (RVFV *Bunyaviridae, Phlebovirus*) is primarily transmitted through mosquito bites, but can also be transmitted by exposure to infectious aerosols. There are presently no licensed vaccines or therapeutics to prevent or treat severe RVFV infection in humans. We have previously reported on the activity of favipiravir (T-705) against the MP-12 vaccine strain of RVFV and other bunyaviruses in cell culture. In addition, efficacy has also been documented in mouse and hamster models of infection with the related Punta Toro virus. Here, we characterize a hamster RVFV challenge model and use it to evaluate the activity of favipiravir against the highly pathogenic ZH501 strain of the virus. Subcutaneous RVFV challenge resulted in substantial serum and tissue viral loads and caused severe disease and mortality within 2-3 days after infection. Oral favipiravir (200 mg/kg/day) reduced viral loads and prevented mortality in 80% of hamsters challenged with RVFV when administered within 1 h post-exposure, whereas only 20% of animals treated with ribavirin (75 mg/kg/day) survived. Ongoing and future studies should reveal further insights into the protective capacity of favipiravir against RVFV.
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169 Inhibition of Chikungunya Virus Replication by T-705 (favipiravir) and Identification of Resistance Associated Mutations in the RNA-dependent RNA Polymerase

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Chikungunya virus (CHIKV) is a mosquito-borne, emerging human pathogen that causes a debilitating, often persistent arthralgia. T-705 (favipiravir), is in development for the treatment of influenza and has demonstrated antiviral activity against different RNA viruses. Here, we describe the antiviral activity of T-705 on CHIKV replication and provide proof that a mutation in the RNA-dependent RNA polymerase (RdRp) gene is responsible for phenotypic resistance to the compound.

T-705 was found to inhibit the *in vitro* replication of a panel of laboratory strains and clinical isolates of CHIKV with EC_{50}s in the range of 2-25 µM (CC_{50} >600 µM). The compound was next evaluated in AG129 mice infected with CHIKV strain S27. Oral administration of T-705 (300 mg/kg/day) reduced virus-induced mortality by 85% and 65% when given pre- or post-infection respectively. Three CHIKV strains, selected independently in the presence of T-705, proved ~2-fold less susceptible to the antiviral activity of the compound. Mutation K291R was observed in the RdRp gene of all three strains. Reverse-engineering of this mutation confirmed that this mutation is responsible for the drug-resistant phenotype. When the strains were further passaged with the compound additional mutations were selected and a further decrease in susceptibility to the compound was observed (~9-fold). A possible association between these additional mutations and resistance is currently being investigated. To our knowledge this is the first report showing that resistance to T-705 resistance can be selected and the thus also the first proof that the viral polymerase is, in the context of the intact infected cell, the target of the drug. [Funded by EU FP7 Program SILVER (n° 260644)].

170 The ATP-specific Priming Site of Dengue Virus Polymerase – a Target Site for the Development of Antivirals

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The *Flavivirus* genus of positive-strand RNA viruses contains important human pathogens such as yellow fever, West Nile, Japanese encephalitis and dengue virus (DV). The genome ends of flaviviruses are strictly conserved as 5›-AG…CU-3›. We demonstrate here the primary role of the DV polymerase in the conservation of the first and last genomic residue. We show that the DV polymerase contains an ATP-specific priming site, which imposes a strong preference for *de novo* synthesis of a dinucleotide primer starting with an ATP. Using mutants we show that a predicted priming loop and, more specifically, loop residue His798 provide at least part of the ATP-specific priming site. This site represents a specific target site of ATP analogs. Efforts at the Antiviral drug discovery platform (AD2P) are underway to exploit *de novo* initiation tests that allow the identification of inhibitors acting at the ATP-specific binding site. We also introduce other replicase activity – based projects of the AD2P aiming at the development of anti-flavivirus inhibitors.

171 Generation and Characterization of Neutralizing Human Recombinant Antibodies Against Antigenic Site of Rabies Virus Glycoprotein

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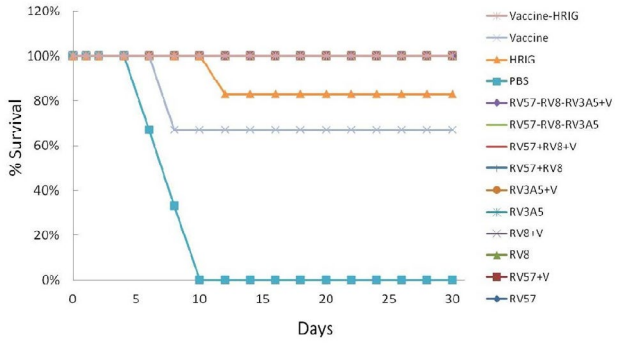
Currently recommended treatment for individuals exposed to rabies virus (RV) is post-exposure prophylaxis (PEP) through the combined administration of rabies vaccine and rabies immune globulin (RIG). Human monoclonal antibodies (mAbs) that neutralize RV offer an opportunity to replace RIG for rabies PEP. Here a combinatorial human Fab library was constructed using antibody genes derived from the blood of RV-vaccinated donors. Selections of this library against purified RV virions resulted in the identification of 11 unique Fab antibodies specific for RV glycoprotein. Of the Fab antibodies, five were converted to full human IgG1 format. The human IgG antibodies revealed high binding affinity and neutralizing activities against RV fixed strains through a rapid fluorescent focus inhibition test (RFFIT) *in vitro* as well as the early stage protective function after exposure to RV infection *in vivo*. Furthermore, epitope mapping and binding competition analysis showed that all of obtained human neutralizing and protective antibodies were directed to the antigenic site Π of RV glycoprotein. Our results provide not only important insight into the protective immune response to RV in humans, but also more candidates eligible for use in a mAb cocktail aimed at replacing RIG for rabies post-exposure prophylaxis.



172 Binding of Glutathioneto Enterovirus Capsids is Essential for Virion Morphogenesis and Depletion of Glutathione Results in an Antiviral Effect

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**equal contribution*

TP219 [9-(3-acetylphenyl)-6-chloropurine] was identified as a selective inhibitor of the *in vitro* replication of several enteroviruses. We demonstrate that the compound interferes with the morphogenesis of progeny virus without affecting early steps in the replication cycle, RNA replication or polyprotein translation/processing. Fractionation studies on sucrose gradients revealed that TP219, like L-BSO (a known enzymatic inhibitor of glutathione synthesis and enterovirus morphogenesis) was essential for the transition of protomers into pentamers and subsequently higher order particles. We next demonstrated that TP219 covalently binds glutathione, causing a rapid depletion of intracellular glutathione levels. Addition of exogenous glutathione, but not dithiothreitol or N-acetyl cysteine, increased virus infectivity in a dose-dependent manner, thus confirming a role of glutathione in the virus morphogenesis. To identify its viral target, TP219-resistant CVB3 variants were selected and were found to carry several mutations in VP1 (T77M, V150I and N212S) and VP3 (A180T) at the interface of two protomers. Using reverse genetics, we demonstrated that only the mutant carrying a methionine at position 77 rendered the virus capable of replicating in the absence of glutathione. Using immunofluorescence, we confirmed that the drug-resistant variants were able to encapsidate independently from glutathione. Finally, biochemical analysis and heat inactivation experiments provided strong evidence for a direct interaction between glutathione and the viral capsid. Therefore, we propose a mechanism whereby glutathione functions as a stabilizing cofactor in the morphogenesis of virus progeny at the protomeric interface.

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173 A Novel Class of Highly Potent Small Molecule Inhibitors of Entero/Rhinovirus Replication that Target the Non Structural Protein 2C

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A series of highly potent inhibitors of entero- and rhinovirus replication was developed. Analogue KR-22809 was used for mechanistic studies. This molecule was shown to inhibit viral RNA replication without affecting polyprotein processing. To identify the viral target, KR-22809 resistant CVB3 variants were selected and were found to carry four amino acid mutations throughout the three conserved motifs of the NTPase domain in the nonstructural protein 2C. The mutations were engineered, either alone or combined, into a full-length CVB3 clone. Only a combination of four mutations conferred full resistance. Resistant virus proved cross-resistant to other earlier-reported 2C-targeting compounds. KR-22809 inhibited the ATPase activity of purified protein CBV3 2C maximally by 60% at relatively high concentrations. Therefore, inhibition of NTPase activity of 2C is likely not responsible for the potent antiviral effect of this class of compounds. Since no functional activity of the helicase activity of 2C is available, the effect of the compound on this activity could not be assessed. To obtain a spatial view on the location of the identified mutations, a model of protein 2C was build based on homology with the Large T antigen of simian virus 40. Localization of the mutations responsible for the drug-resistance revealed that these residues, although widely spread over the primary amino acid sequence, are located in close proximity to each other when considering the tertiary structure of the protein. We therefore hypothesize that KR-22809 inhibits helicase activity or the assembly of the putative helicase into a functional entity. We here report for the first time that protein 2C is an excellent target for the development of potent (low nM) and broad-spectrum inhibitors of enterovirus replication with a high barrier to resistance. Supported by EU FP7 project SILVER (grant 260644)

174 Synthesis and Antiviral Activities of Hexadecyloxypropyl Prodrugs of Acyclic Nucleoside Phosphonates Containing Guanine or Hypoxanthine and a (S)-HPMP or PEE Acyclic Moiety

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Hexadecyloxypropyl esters of acyclic nucleoside phosphonates containing guanine (G) or hypoxanthine (Hx) and a (*S*)-[3-hydroxy-2-(phosphonomethoxy)propyl] [(*S*)-HPMP] or 2-(2-phosphonoethoxy)ethyl (PEE) acyclic moiety have been prepared. The activity of the prodrugs was evaluated *in vitro* against different virus families. None of the hexadecyloxypropyl esters of (*S*)-HPMPHx nor PEEHx proved active against herpesviruses and poxviruses. Although the hexadecyloxypropyl diester of PEEG was antivirally inactive, the monoester derivative gained activity against herpesviruses. Attachment of highly lipophilic hexadecyloxypropyl moieties to (*S*)-HPMPG resulted in great improvement of its antiviral activity against herpesviruses. The cyclic monoester derivative of (*S*)-HPMPG emerged as the most selective antiviral compound. None of the compounds were inhibitory against RNA viruses and retroviruses.

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175 Development of a High-Throughput Screening Platform for BSL-4 Pathogens

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A large portion of antiviral drug discovery begins with a high-throughput screening (HTS) campaign against the pathogen or targets of interest. In the case of whole, wild-type pathogen screening, modern biosafety requires the adaptation of HTS operations to a biocontained environment appropriate for the specific pathogen. Southern Research Institute has successfully performed extensive HTS (>100,000) campaigns at Biosafety Level 2 (BSL-2) and (BSL-3), but HTS with BSL-4 viruses (e.g., Nipah or Ebola viruses) presents greater challenges, due to restricted operations in a BSL-4 laboratory. To circumvent this, many of the current HTS efforts towards BSL-4 viruses employ pseudotype, surrogate, or recombinantly-attenuated viruses that have been downgraded for use at lower biocontainment levels, with the caveat that these efforts must be repeated with the wild-type virus at BSL-4 to ensure that the surrogate virus maintains predictive fidelity. As a result, potential hits inhibiting other steps in the replication cycle are entirely missed in a pseudotyped virus-based screening. Southern Research Institute and the University of Texas Medical Branch (Galveston National Laboratory) have established a collaborative platform for BSL-4 HTS, wherein Southern Research Institute provides HTS expertise, compound libraries, and bioinformatics support, and the University of Texas Medical Branch provides laboratories, pathogens, and pathogen expertise. This platform is specifically designed to screen up to 10,000 compounds/day to identify prophylactic and post-exposure therapeutic candidates against BSL-4 pathogens, particularly Nipah, Ebola, Marburg, and Lassa viruses. Jointly, we have conducted a pilot screen with 10,000 compounds and, confirmed actives in a 10 point dose response against Nipah virus. Compound toxicity was also evaluated using the same cell line as the antiviral screen. From this pilot effort, 23 compounds were identified with IC_{50} values ranging from 2-20 micrograms/ml and selectivities > 10. This platform will be expanded to include additional BSL-4 level pathogens.

176 Drug Design Studies on DENV RdRp

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The mosquito-borne dengue virus (DENV), belonging to the Flaviviridae family, causes approximately 50-100 million new infections in humans every year and it is endemic in over 100 countries. Three distinct clinical pictures have been described for these infections: dengue fever, dengue haemorrhagic fever and dengue shock syndrome. The latter two have a more frequent fatal outcome and are commonly caused by a secondary infection with a different DENV serotype. Nevertheless, there is currently no specific antiviral treatment available and, to date, vaccine development has proven very difficult. The viral single-stranded, positive-sense RNA genome encodes three structural and seven non-structural (NS) proteins. The latter are viral enzymes and cofactors, and therefore attract significant attention for the development of selective inhibitors of virus replication. As it is essential for genome replication, NS5 RNA-dependent RNA polymerase (RdRp) is one of the most promising drug targets. Starting from the RdRp incomplete crystallographic structure, we have modelled in silico the missing portions using the West Nile Virus RdRp as a template. On the obtained model, molecular modelling techniques were applied to investigate an allosteric binding site of NS5 for the design of potential inhibitors of this protein. For initial *in vitro* evaluation, a virtual screening approach was implemented for the selection of drug-like compounds from a database of approximately 210,000 molecules. Analogues of the selected compounds were then synthesized. In this presentation, we will discuss the preliminary results obtained and the potential implications in antiviral drug design.

177 Novel Prodrugs of Acyclovir Cleavable by the Dipeptidyl Peptidase IV (DPP IV/CD26) Enzyme

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The lymphocyte surface glycoprotein dipeptidyl-peptidase IV enzyme (DPP-IV/CD26), selectively cleaves X-Pro (or X-Ala) dipeptides from the N-terminus of a variety of natural peptides. We have developed an entirely novel enzyme-based prodrug approach that provides conjugates of therapeutic agents with a di- (or oligo) peptide moiety as a carrier, wherein the conjugate [peptide]-[drug] is specifically cleavable by the endogenous DPP-IV/CD26.(1) The approach was first applied to a variety of drugs containing a free amino group that was directly coupled with the carboxyl group of amino acids via an amide bond (*bipartate prodrugs*). It was possible to modify the hydrolisis rate (half-life) and the physicochemical properties of the compounds by modifying the nature and length of the peptide (di- or tetrapeptides).(2) In order to apply our prodrug strategy to hydroxy-containing drugs, we recently designed [Xaa-Pro]-[connector]-[drug] conjugates (*tripartate prodrugs*) bearing heterobifunctional connectors (i.e. amino acids or self-cleavage linkers) to link the peptide moiety to the hydroxyl group of the drug. Several of the synthesized prodrugs showed a high improvement in aqueous solubility and oral bioavailability together with an efficient conversion to the biologically active parent drug in the presence of DPPIV/CD26 in a variety of biological media.(3) We now explore the viability of this DPP-IV/CD26 prodrug approach in the antiviral acyclovir. Prodrugs bearing peptide promoieties at the amino group of the guanine base or at the hydroxyl group of the acyclic chain have been evaluated for their pharmacokinetic properties including chemical and enzymatic stability (cleavage rates) and water solubility. Solution and solid-phase N-acylation protocols of the exocyclic amino function of the guanine base will be described. REFERENCES: *(1) García-Aparicio, C. et al. J. Med. Chem. 2006, 49, 5339; (2) Diez-Torrubia, A. et al. Antivir. Res. 2007, 76, 130 J. Med. Chem. 2010, 53, 559; (3) Diez-Torrubia, A. et al. J. Med. Chem. 2011, 54, 1927 ChemMedChem 2012, 7, 618-628 ChemMedChem 2012, 7, 1612-1622.*

178 Human Cytomegalovirus Resistance to Cyclopropavir Maps to a Base Pair Deletion in UL97 Which Results in a Viral Protein Lacking an Active Kinase Domain

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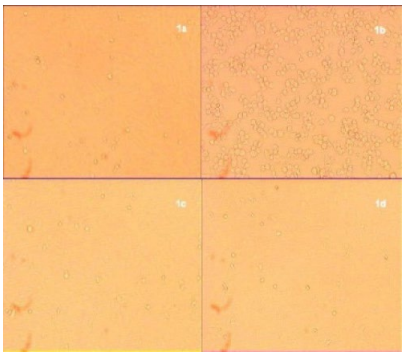
Human cytomegalovirus (HCMV) is a widespread pathogen in the human population affecting many immunologically immature and immunocompromised patients, and can result in severe complications such as interstitial pneumonia, hearing loss, and mental retardation. Current chemotherapies for the treatment of systemic HCMV infections include ganciclovir (GCV), foscarnet, and cidofovir. However, high incidences of adverse effects (neutropenia and nephrotoxicity) are prevalent and limit the use of these drugs. Cyclopropavir (CPV), a guanosine nucleoside analog, is 10-fold more active (EC_{50} = 0.46 uM) than GCV (EC_{50} = 4.1 uM) without any observed increase in cytotoxicity. We hypothesize that the mechanism of action is similar to GCV namely phosphorylation to a monophosphate by viral pUL97 protein kinase and further phosphorylation to a triphosphate by endogenous kinases resulting in incorporation into HCMV DNA and inhibition of viral DNA polymerase. To test the first step of this hypothesis, we isolated a CPV-resistant virus, sequenced the drug resistant viral genome, and discovered that base pair 498 of *UL97* was deleted. This mutation caused a frame shift in *UL97* resulting in a truncated protein that lacks a kinase domain. To determine if this base pair deletion is responsible for drug resistance, the mutation was engineered into the wild-type viral genome and resulting virus was then subjected to increasing concentrations of CPV. The results demonstrate that the engineered virus was approximately 170-fold more resistance to CPV (EC_{50} = 42.2 uM) when compared to wild-type virus (EC_{50} = 0.25 uM). We conclude therefore that this mutation is necessary and sufficient for drug resistance and that pUL97 is involved in the mechanism of action of CPV.

179 Efficacy of *Sida Cordifolia* L. Extracts Against Herpes Simplex Virus Type I Infection *In vitro* and *In vivo*

Ashish Wadhwani[1], Viral Patel[1], Kurokawa Masahiko[2], Vijayan Pottekad[1]
[1]*Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ootacamund, India,* [2]*Department of Clinical Pharmacology, Kyushu University of Health and Welfare, Nobeoka, Japan*

INTRODUCTION: There is an increasing need for substances with antiviral activity since the treatment of viral infections with the available antiviral drugs often leads to the problem of viral resistance. Extracts of *Sida Cordifolia* L. were investigated for potential antiviral effect against Herpes Simplex Virus Type I (HSV-I) *in vitro* and *in vivo* and efforts had also been taken to study the underlying mode of action. MATERIALS & METHODS: The whole plant of *Sida cordifolia* was extracted with different non polar to polar solvents. These extracts were screened for their cytotoxicity against Vero cell lines by Microculture tetrazolium (MTT) and Sulphorhodamine-B (SRB) assays. The antiviral effect of the plant extracts on HSV-I were tested in infected Vero cells by MTT antiviral assay, plaque reduction assay and mechanism based studies were carried out by virus adsorption, penetration and replication assay. The therapeutic efficacies were characterized using a cutaneous HSV-1 infection in mice. **Results:** The hydro alcoholic extract reduced viral infectivity by 96 ± 1.02% against HSV-I (figure 1) when challenged with highest virus challenge dose (100 $TCID_{50}$). The toluene extract showed the presence of phytosterols produced 95 ± 1.22 % cell protection. All the extracts showed dose dependent activity with viral strain. The toluene extract at a dose of 750 mg/kg per day significantly delayed the development of skin lesions ($P<0.05$), prolonged the mean survival times and reduced the mortality of HSV-I wild type 7401 virus infected mice as compared with 1% DMSO in distilled water ($P<0.001$). There was no significant difference between acyclovir and this extract in delay in development of skin lesions. Toxicity of these plant extracts were not observed in treated mice. CONCLUSION: Present investigation finding reveal that the toluene extract of *Sida cordifolia* L. have good antiviral potential and may be possible candidates of anti HSV-I agents. Further in depth studies on anti HSV-1 activity are progressing in our laboratory.



KEYWORDS: Cytotoxicity, HSV-I, MTT assay, *Sida Cordifolia L.*, SRB assay, $TCID_{50}$

180 Immunopathogenesis of Different Common Emerging Infections: Enterovirus 71, Dengue Hemorrahgic Fever, SARS and A (H1N1)2009 Influenza

Kuender D. Yang[1], Lin Wang[2], Jien-Wei Liu[2], Ron-Fu Chen[1], Chun-chen Li[2]
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An emerging infection usually evolves from a mutant virus or from a zoonotic and/or vector-borne transmission, and causes a high fatality due to disseminated viremia, overt inflammation, tissue-cytotropic damage, hemorrahgic fever or even iatrogenic complications.Clarification of the immunopathogenesis of different emerging infections can provide a plan for the crisis management and prevention of an emerging infection. We encountered 4 emerging infections: enterovirus 71 (EV71) encephalitis, dengue hemorrahgic fever, SARS and A (H1N1)2009 influenza in Taiwan in the past decade. Employing a simultaneous measurement of virus load and immunity in blood, we studied and summarized immunopathogenesis of the 4 emerging infections below. 1) EV71 encephalitis was related to children's naïve immunity with lower CD40L expression and viremia (Yang, et al. *J Infect Dis* 2001; 183:850-6.; Li & Yang, et al. *Scand J Infect Dis* 2002; 34:104-9). 2) SARS was manifestated with earlier immunosuppression, followed by later augmented immunity (Lee & Yang, et al. *J Immunol* 2004; 172:7841-7.; Chen & Yang, et al. *BMC Genomics* 2005; 6:132). 3) Dengue hemorrahgic fever (DHF) was correlated to immune augmentation but not viremia (Chen & Yang, et al. *Trans R Soc Trop Med Hyg 2007; 101:1106-13.;* Wang & Yang, et al. *Am J Trop Med Hyg 2008; 79:149-53)*, and associated with C-type lectins (CD209 and CD299) polymorphisms (Wang & Yang, et al. *PLoS Negl Trop Dis 2011; 5:e934)*. 4) A(H1N1)2009 influenza was related to age-dependent virus shedding time (Li & Yang et al. *Emerg Infect Dis* 2010; 16:1265-72.; Chu & Yang, et al. *PLoS One* 2012; 7:e32731), and depressed innate immunity with augmented Treg function. Based on immunopathogenesis of these 4 emerging infections,

we formulate a guide to predict and prevent EV71 encephalitis by measuring CD40L levels and viral load, to treat SARS patients in early and late phase differently, to prevent DHF by detecting C-type lectin polymorphisms and augmented immunity, and to contain A(H1N1)2009 influenza by early anti-virus treatment for limiting children's viral shedding and avoiding secondary infection.

ORAL SESSION 2: HEPATITIS AND HIV

Chairs: William Delaney, Ph.D. and Dirk Daelmans, Ph.D.

2:15 – 4:30 pm

BALLROOM BC

16 Discovery of 6-(indol-2-yl)pyridine-3-sulfonamides as Novel HCV Inhibitors Targeting the Viral NS4B

Gary M. Karp, Xiaoyan Zhang, Nanjing Zhang, Guangming Chen, Anthony Turpoff, Neil Almstead, Zhengxian Gu, Joseph Colacino
PTC Therapeutics, South Plainfield, USA

Recently, the first direct-acting antiviral agents, the HCV protease inhibitors Victrelis® and Incivek® were approved for the treatment of HCV genotype 1 infection. Several additional compounds are in preclinical or clinical development targeting the viral proteins NS3, NS5A, and the RNA-dependent RNA polymerase NS5B. Here we report the discovery of a series of novel compounds that target the HCV NS4B to inhibit the replication of HCV RNA. Starting from a series of N-(4'-(indol-2-yl)phenyl sulfonamides, we determined that reversal of the sulfonamide linkage led to compounds with improved potency against the HCV genotype 1b replicon. Replacement of the indole 2-phenyl group with a pyridyl ring led to a series of highly potent and selective 6-(indol-2-yl)pyridine-3-sulfonamides. Preliminary optimization of this series furnished compounds with potency in the low nanomolar range against the HCV genotype 1b replicon. The sulfonamide group was found to be susceptible to metabolism and modification of both the indole 2-arene and sulfonamide moieties resulted in the identification of compounds with improved oral exposure, leading to the discovery of PTC-512, a highly potent analog with excellent pharmacokinetic properties in rats. PTC-512 has potent inhibitory activity (EC_{50} = 4 nM) with a selectivity index with respect to cellular GAPDH RNA of more than 2,500. Further, PTC-512 has an IV half-life of 6 hours and oral bioavailability (F) of 62% in rats. Selection of HCV replicon resistance identified an amino acid substitution in the NS4B that confers resistance to these compounds. This series hold promise as a new chemotype with anti-HCV activity mediated through an underexploited viral target.

17 Next-generation HCV NS5A Inhibitor: *In vitro* Antiviral Optimization for Pan-genotypic Activity and Preclinical Rrofile

Cyril B. Dousson[1], Christophe C. Parsy[1], David D. Dukhan[1], Jean-L. Paparin[1], Francois-R. Alexandre[1], Maria Seifer[2], Xin-R. Pan-Zhou[2], David N. Standring[2]
[1]Idenix Pharmaceuticals, Montpellier , France, [2]Idenix Pharmaceuticals, Cambridge, USA

BACKGROUND: This report presents the discovery effort of novel, pan-genotypic next-generation inhibitors of NS5A, a protein essential for HCV replication and virion production via multiple functions. METHODS: The structure of the five main parts of the NS5A inhibitors were modified individually to optimize their activities. The antiviral activities and specificities of the NS5A inhibitors were assessed in standard assays utilizing HCV replicons of different genotypes. The pharmacokinetic profiles of the best inhibitors were determined by standard methods in rats and cynomolgus monkeys. RESULTS: Extensive optimization of structurally diverse NS5A inhibitors led to the selection of a restricted number of promising scaffolds. In this process, the modification of the most distal part of the inhibitor was identified as having the largest impact on maintaining genotype (GT) 1a activity. Other modifications proved to have an impact on the activity of all GTs, and the combination of the best central scaffold with desymmetrization of the distal parts was the key to achieving picomolar pan-genotypic activity. In GT 1a to 5a, the selected candidate IDX719 exhibited replicon EC50s from 2 to 24 pM and did not show toxicity at the highest

concentration tested (100 mM), yielding a selectivity index of >33 million in Huh7 cells. Oral administration of IDX719 (10 mg/kg) in the cynomolgus monkey and mouse led to C_{24} trough levels some 1000- to 3000-fold above the preclinical EC_{50} values for the different genotypes, consistent with the robust antiviral activities demonstrated in genotypes 1-4 in the clinic. CONCLUSIONS: The combination of the most appropriate central scaffold and distal part modification allowed the discovery of pan-genotypic low picomolar NS5A inhibitors. IDX719, our lead NS5A inhibitor, has demonstrated potent, pan-genotypic antiviral activity in a 3-day proof-of-concept study in HCV-infected subjects in genotypes 1-4. The pharmacokinetic profile of IDX719 in human subjects supports a once-daily dosing regimen.

18 Identification of PTC725: A Potent, Selective and Orally Bioavailable Small Molecule that Targets the Hepatitis C Virus NS4B Protein

Jason D. Graci, Zhengxian Gu, Stephen P. Jung, Gary Karp, Neil G. Almstead, Joseph M. Colacino
PTC Therapeutics, Inc., South Plainfield, USA

We have identified a novel class of potent HCV inhibitors, exemplified by PTC725, that target the nonstructural protein 4B (NS4B). PTC725 inhibited HCV 1b (Con 1) replicon with an EC_{50} of 1.7 nM and an EC_{90} of 9.6 nM and demonstrated a >1000-fold selectivity window with respect to cytotoxicity. The compounds were fully active against HCV replicon mutants that are resistant to inhibitors of NS3 protease and NS5B polymerase. Replicons selected for resistance to PTC725 harbored amino acid substitutions F98L/C and V105M in NS4B. Anti-replicon activity of PTC725 was additive to synergistic in combination with alpha interferon or with inhibitors of HCV protease and polymerase. Immunofluorescence microscopy demonstrated that neither the HCV inhibitors nor the F98C substitution altered the subcellular localization of NS4B or NS5A in replicon cells. Oral dosing of PTC725 showed a favorable pharmacokinetic profile with high liver and plasma exposure in mice and rats. Modeling of dosing regimens in humans indicates that a once-per-day or twice-per-day oral dosing regimen is feasible. Currently, PTC725 is under evaluation in IND enabling studies in collaboration with the NIH. Preclinical data support the continued development of PTC725 for use in the treatment of chronic HCV infection.

19 Flexible Nucleotides as Antivirals

H. Peters[1], H. Senderowitz[2], K. Seley-Radtke[1]
[1]*University of Maryland Baltimore County, Baltimore, USA,* [2]*Bar-Ilan University, Ramat-Gan, Israel*

The pool of potent antiviral drugs is constantly shrinking due to the rise of drug resistance. This project explores how novel base flexibility, when combined with active nucleotide antiviral sugar scaffolds, affects viral polymerase (HIV, HSV, HCV, HBV...) binding and function through both in-vitro and computational studies. It is predicted that these analogs will possess higher binding affinity along with the capability of overcoming point mutations in the polymerase active site that previously conferred resistance. The need for new and more potent antiviral therapeutics is critical due to increasing reports of drug resistance, as well as emerging new viral diseases. It has been shown that Etravirine, an FDA-approved flexible heterobase analog, can adapt conformationally and positionally to resistance mutations encountered in the HIV reverse transcriptase (RT) binding site. This flexibility allows it to retain potency against resistant strain. Similarly, a "split base" guanosine triphosphate analog developed in our laboratory retained full potency against binding site mutations in guanosine fucose pyrophosphorylase (GFPP) due to interactions with secondary amino acid residues not previously involved with the mechanism of action. As a result, exploitation of conformational and positional flexibility in the nucleobase scaffold can be viewed as a powerful tool for developing drugs that can retain their effectiveness against Recently, in collaboration with Dr. Senderowitz (Bar-Ilan University) we carried out preliminary MD simulations on tenofovir and flex-tenofovir in the binding site of HIV-RT complexed with DNA. The RMSD results suggest that flex-Tenofovir is better able to explore the binding site, and does in fact use its increased flexibility to sample the binding site better. Measured differences in the centroid-centroid distance also predict that our analog is capable of exploring more regions within the binding site. The enthalpic term is also predicted to be more favourable for our flex analog. We predict a greater number of H-bonds for our analog over the natural substrate and Tenofovir. These results are highly encouraging and strongly support further computational investigations of the effects of substrate flexibility on viral polymerase fidelity.

20 Replication Inhibition by Small-molecules Targeting the HIV Rev-CRM1 Interaction

Eline Boons[1], Thomas Vercruysse[1], Sharon Shacham[2], Yosef Landesman[2], Erkan Baloglu[2], Sharon Tamir[2], Christophe Pannecouque[1], Dirk Daelemans[1]
[1]Rega Institute, KU Leuven, Belgium, [2]Karyopharm Therapeutics, Natick, USA

The HIV-1 Rev protein is essential for viral replication as it directs the transport of late viral mRNAs to the cytoplasm. Rev multimerizes on the viral mRNA and interacts with the cellular transport factor CRM1 through its nuclear export signal. CRM1 on its turn guides the Rev-mRNA complex to the cytoplasm where the complex is dissociated and the viral mRNAs can serve as templates for viral structural protein synthesis or as viral genome. The interaction of Rev with this cellular co-factor CRM1 is essential for the expression of the late viral mRNAs and for viral replication, and is therefore a candidate target for therapeutic anti-HIV strategies. We have now designed a drug-like small-molecule inhibitor of the Rev-CRM1 protein-protein interaction. This molecule inhibits the replication of both wild-type and drug-resistant HIV-1 strains and also suppresses the replication of clinical isolates at nanomolar concentrations with good selectivity in primary cells. The compound is orally bioavailable and displays good pharmacokinetics in mice, rats, dogs and monkeys. Mechanism of action studies demonstrate that it prevents the nuclear export of viral RNA in the infected cells. These results validate the interaction between the cellular host factor CRM1 and the viral protein Rev as promising target for a novel anti-HIV intervention approach but also open new perspectives for the treatment of other viral infections involving CRM1-mediated nuclear export.

21 Evolution of Pyrimidinedione HIV Therapeutic Agents with Improved Solubility and Metabolic Stability

Robert W Buckheit, Jr., Karen W Buckheit, Anthony Ham, Tracy Hartman
ImQuest BioSciences, Inc., Frederick, USA

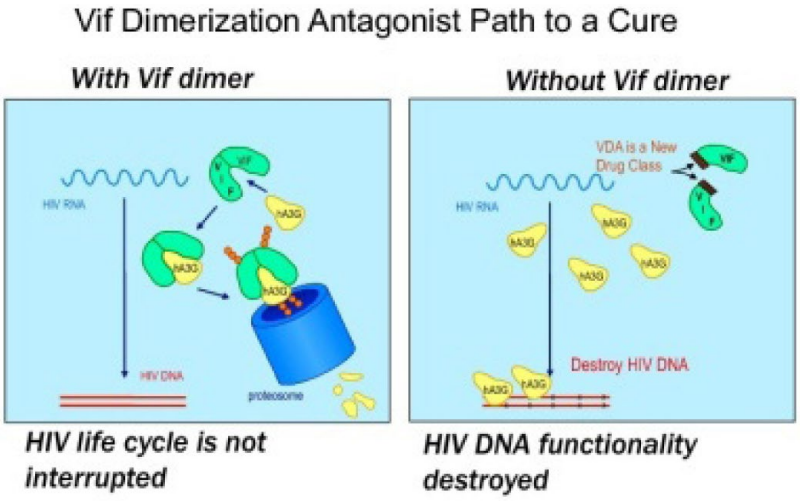
The pyrimidinediones are highly potent nonnucleoside inhibitors of both HIV-1 and HIV-2, inhibiting both RT and virus entry at nanomolar concentrations, including all HIV subtypes (except subtype O) and MDR strains. Our initial lead clinical candidate (IQP-0410) was subjected to a comprehensive program of standard investigational new drug (IND)-enabling GLP studies in order to establish the acute and multiple dose toxicity, toxicokinetics, genotoxicity, and safety pharmacology profile. IQP-0410 was rapidly metabolized by first pass hepatic metabolism. The primary development hurdles associated with IQP-0410 (solubility and metabolic stability) were chemically addressed and a new lead (IQP-0528) was identified and evaluated. The N_1-cyclopentenyl group of IQP-0410 undergoes multiple oxidations; modification of this substituent to N_1-cyclopropyl (IQP-0528) yielded significantly greater stability without loss of antiviral efficacy or enhanced toxicity. With IQP-0528 the major metabolic pathway is oxidation of one of the methyls of the C6-linked $C_6H_4Me_2$ group, as has been reported for the metabolism of other uracil-based NNRTIs, such as Emivirine (MK-442). Replacement of the 3,5-dimethylphenyl with 3,5-dichloro, resulted in the highly stable compound IQP-1960 (JDJ-01). IQP-1960 was found to be as potent as IQP-0410 and IQP-0528 against clinical subtypes of HIV-1 and possessed superior pharmacokinetic properties. As lead therapeutic (IQP-1960) and prevention (IQP-0528) agents, the comparative preclinical pharmacology, including range of antiviral activity, tissue sterilizing concentrations, combination use and resistance selection will be reported for each compound. Both compounds are active against all subtype viruses at nanomolar concentration levels, inhibit both reverse transcription and virus entry, and act in an additive to synergistic manner with all other approved antiretroviral agents. *In vitro* resistance selection highlights the need for an accumulation of multiple mutations in the RT and Env (gp120 and gp41) to achieve high level resistance. IQP-1960 is highly stable and preformulation studies suggest the molecule can be effectively delivered either orally or through a transdermal film.

22 A Novel Class of Antivirals for HIV/AIDS Intervention Revealed by Targeting the HIV Vif Protein Dimerization Domain

Harold/C Smith[1,2], Ryan/P Bennett[2]

[1]*Dept. Biochemistry University of Rochester, Rochester, USA,* [2]*OyaGen, Inc, Rochester, USA*

The HIV auxiliary protein known as Viral infectivity factor (Vif) enables HIV to overcome cellular APOBEC host-defense factors by binding to these proteins as a substrate receptor, thereby ensuring their ubiquitination and degradation through the 26S proteasome. Vif-Vif interaction through the PPLP domain has been shown to be 'druggable' in living cells and disruption of Vif dimers inhibited live virus in spreading infection assays by protecting A3G from Vif-dependent degradation and enabled A3G incorporate in viral particles (Miller et al., (2007) Retrovirology 4:81-91). Live cell FRET for Vif dimerization in high throughput screening together with medicinal chemistry has identifying a small molecule Vif dimerization antagonist (VDA) with low cytotoxicity and nM antiviral efficacy. Inhibition of HIV infection showed strict dependence on the expression of Vif and APOBEC3G. VDA enabled A3G antiviral activity by protecting A3G from Vif-dependent degradation, enhanced virion incorporation of A3G and inhibited viral replication. Compounds that bind to Vif and inhibit Vif dimerization underscore the need to aggressively pursue this target for first-in-class therapeutics, prophylaxis and in combination with A3G activators, a potential cure.



ORAL SESSION 4: INFLUENZA AND RESPIRATORY INFECTIONS

Chairs: Bruno Canard, Ph.D. and Gerhard Puerstinger, Ph.D.

1:30 – 4:30 pm

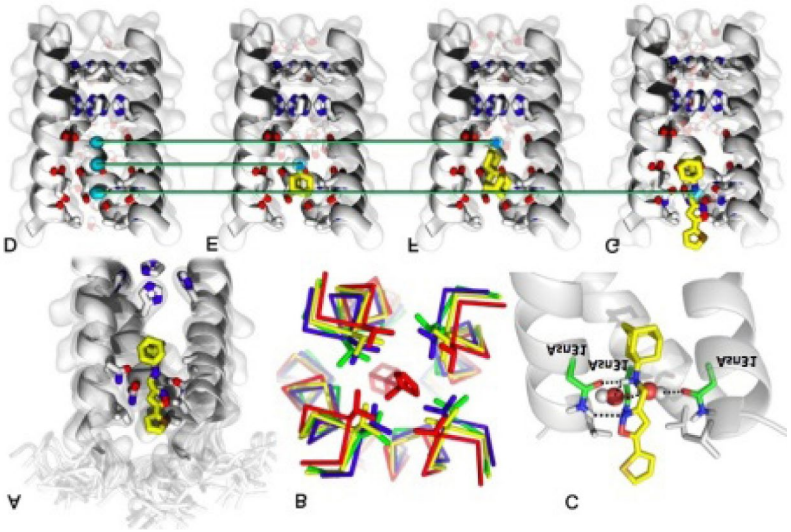
BALLROOM BC

116 Structure and Inhibition of the Drug-resistant mMutants of the M2 Ion Channel of Influenza A Virus

Jun Wang[1], Yibing Wu[1], Chunlong Ma[2], Robert Lamb[2], William DeGrado[1]

[1]UCSF, San Francisco, USA, [2]Northwestern University, Evanston, USA

The influenza A virus M2 proton channel (A/M2) is the target of the antiviral drugs amantadine and rimantadine, whose use has been discontinued due to widespread drug resistance. Among the handful of drug-resistant mutants, S31N, V27A and L26F are found in more than 99% of the currently circulating viruses and shows greatly decreased inhibition by amantadine. The small set of transmissible mutants argues that M2 is a highly conserved drug target comparing with other viral proteins, rendering it an ideal target for anti-flu drug development. The discovery of inhibitors of these M2 mutants has been hampered by the the lack of structural information and their limited sizes, polarity, and dynamic nature of their drug binding sites. Nevertheless, using an integrated approach including medicinal chemistry, molecular dynamics simulation, solid/solution-state NMR, X-ray crystallography, and pharmacological characterizations, We have discovered small molecule drugs that inhibit S31N, V27A and L26F with potencies greater than amantadine's potency against WT M2 in two electrode voltage clamp assay. The activities of potent compounds were further confirmed in viral replication assays. A few compounds with ~100 nM EC_{50}s are advanced to mice model studies. Structural characterization of S31N drug binding by NMR shows the drug bound in the homotetrameric channel, threaded between the side chains of Asn31. This S31N inhibitor, like other potent M2 inhibitors, contains a charged ammonium group. The ammonium binds as a hydrate to one of three sites aligned along the central cavity that appear to be hotspots for inhibition. This model of M2 drug inhibition could be similarly applied to design other channel blockers.



117 New Small Molecule Entry Inhibitors Targeting Hemagglutinin-mediated Influenza A Virus Fusion

Arnab Basu[1], Michael Caffrey[2], Dale L. Barnard[3], Lijun Rong[2], Terry L. Bowlin[1]

[1]Microbiotix Inc, Worcester, USA, [2]University of Illinois at Chicago, Chicago, USA, [3]5. Institute for Antiviral Research, Utah State University,, Logan,

Influenza viruses are a major public health threat worldwide and options for antiviral therapy are limited by the emergence of drug-resistant virus strains. The influenza glycoprotein hemagglutinin (HA) plays critical roles in the early stage of virus infection, including receptor binding and membrane fusion making it a potential target for developing anti-influenza drugs. Using pseudotype virus based high throughput screens, we have identified several new small molecules capable of inhibiting influenza virus entry. We prioritized two novel inhibitors MBX2329

and MBX2546, with aminoalkyl phenol ether and sulfonamide scaffolds respectively, that specifically inhibit HA-mediated viral entry. The two compounds (a) are potent (IC_{50} = 0.47–5.8 μM), (b) are selective (CC_{50} >100 μM) with selectivity index (SI) values >20-200 for different influenza strains, (c) inhibit a wide spectrum of influenza A virus that includes the 2009 pandemic influenza A/H1N1/2009, highly pathogenic avian influenza (HPAI) A/H5N1, and oseltamivir resistant A/H1N1 strains, (d) exhibit large volumes of synergy with oseltamivir (36- 331 μM2 with 95% confidence) and (e) have chemically tractable structures. Preliminary mechanism of action studies suggest that both MBX2329 and MBX2546 bind to HA in a non-overlapping manners. Additional results from HA-mediated hemolysis of chicken red blood cells (cRBCs), competition assay with MAb C179 and mutational analysis suggest that the compounds bind in the stem region of the HA trimer and inhibit HA mediated fusion. Therefore, MBX2329 and MBX2546 represent new starting points for chemical optimization and have the potential to provide valuable future therapeutic options and research tools to study the HA mediated entry process.

118 Mechanism of Action of Favipiravir (T-705) Revealed by a Novel Biochemical Assay Using Recombinant Influenza A Virus Polymerase

Zhinan Jin[1], Vivek K. Rajwanshi[1], David B. Smith[1], Baek Kim[2], Julian A. Symons[1], Lawrence M. Blatt[1], Leonid Beigelman[1], Jerome Deval[1]

[1]Alios BioPharma, Inc., South San Francisco, USA, [2]University of Rochester Medical Center, Rochester, USA

BACKGROUND: T-705 (favipiravir) is a broad-spectrum antiviral molecule that is in late stage clinical development for the treatment of influenza virus infection. It is believed that T-705 potency is mediated by its ribonucleoside triphosphate (T-705 RTP) metabolite inhibiting the polymerase of influenza A virus (IAVpol). However, the exact molecular mechanism of inhibition has not been elucidated due to the lack of established methods.

RESULTS: We developed enzymatic methods to evaluate the substrate specificity of nucleotide analogs using influenza virus polymerase. We first demonstrated that T-705 RTP inhibited IAV genomic RNA transcription catalyzed by RNA replicase complex extracted from virus. Second, we showed that T-705 RTP only competed with GTP and ATP but not CTP and UTP in an RNA replication assay catalyzed by recombinant IAVpol complex. Finally, we developed a novel enzymatic assay to measure the kinetics of nucleotide incorporation by IAVpol in its elongation mode. Using short synthetic RNA templates with specifically designed sequences, the IAVpol was able to pause or extend the RNA replication by recognizing specific nucleoside triphosphates added into the polymerase reaction. With this assay, T-705 RTP was incorporated both as a GTP and an ATP analog. Concentration dependence of nucleotide incorporation was performed to obtain the apparent substrate specificity. Compared to natural GTP and ATP, the incorporation of T-705 RTP was only 19- and 30-fold less efficient, respectively. Although the single incorporation of the nucleoside monophosphate form of T-705 did not readily block RNA synthesis, two consecutive incorporation events prevented further RNA extension. In comparison, 3'-deoxy GTP caused immediate chain termination but was incorporated about 4,900-fold less efficiently than GTP. **Conclusions:** T-705 RTP can be incorporated into RNA as an ATP and a GTP analog, and causes delayed chain termination. The combination of ambiguous base-pairing with high substrate efficiency of T-705 RTP provides a mechanistic basis for the lethal mutagenesis theory.

119 GP1001, a Host Targeted Influenza Therapy

Dary Faulds[1], Dale Barnard[2], John Morrey[2], Jiing-Huey Lin[1], Hsiao-Lai Liu[1], Bart Tarbet[2], William Guilford[1]

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

The challenge for host-targeted, influenza therapies is to demonstrate efficacy even though such therapies may not reduce the viral load either *in vitro* or *in vivo*. The therapeutic benefit of reducing the high level of viral-induced cytokines is demonstrated in the characterization of the development candidate GP1001 in the mouse lethal challenge influenza model. These results support the promise of host-targeted therapies in reducing the

symptoms of either seasonal or pandemic influenza by reducing the underlying cause of the symptoms. Using BALB/c mice and infecting with the Influenza A/Duck/MN/1525/81 (H5N1) virus, treatment with GP1001 (ip 1.2 mg/Kg/d) at day 0 protected against death compared to placebo mice (50% vs 20% survival) and with an increase in the mean day of death (MDD) to 11.4±1.8 d from 8.4±3.2 d (placebo). A reduction in lung score was seen in GP1001 treatment compared to placebo at day 3 (0.0±0.0 vs 0.9±0.4) and at day 6 (2.6±0.4 vs 3.6±0.3). Similar results were seen with lung weight between the GP1001 and placebo group at day 6 (0.21±0.02 vs 0.35±0.06). The level of pro-inflammatory cytokines at day 6 in the lung of GP1001 treated mice was significantly lower than corresponding level in placebo mice. For example, in GP1001 treated mice the levels of IFNg were 2,200 pg/ml vs 5,100 pg/mL in placebo-treated mice, corresponding values for MCP-1 levels were 3,000 pg/ml vs 13,300pg/mL and for IL-6 the levels were 1,000 pg/ml vs 4,900pg/mL. Histology of thin slices of lungs showed a dramatic decrease in the infiltration by macrophages and neutrophils after GP1001 treatment compared to placebo treated mice. The combination of GP1001 (ip, 1.2mg/kg/d) and oseltamivir (os, 1 mg/kg/d) resulted in complete protection against death, an increase in MDD and improved lung score and weight over placebo. In contrast, there was no significant change in lung viral titers or body weight between GP1001-treated and placebo treated mice. Thus, the ability of GP1001 to significantly reduce the level of inflammation in the lungs of mice translated into an increase in survival without reducing viral titer. Therefore, GP1001 is a promising host-targeted treatment of both seasonal and pandemic influenza infections.

120 Identification of a Potent Fusion Inhibitor of Influenza A Virus

K.K. Lai, F. Yang, K.Y. Yuen, R.Y. Kao
Hong Kong University, Hong Kong SAR, China

Influenza A virus is the major pathogen contributing to seasonal infections and even severe pandemics. Due to the frequent usage of antiviral drugs like Tamiflu and Amantadine, drug resistance virus has appeared rapidly. There is an urge to develop effective vaccines or antivirals for influenza A virus associated infections. In the initial step of viral infection, hemagglutinin (HA) plays a critical role in recognizing the host cells and inducing membrane fusion under acidic pH. Using forward chemical genetics, we have identified a small molecule compound, which effectively inhibited H1N1, H5N1, and 2009 swine-origin influenza A viruses with nanomolar median effective concentration (EC50), from a chemical library with 50,240 structurally-diverse compounds. To uncover the mechanism of the compound, time-of-addition assay was performed and significant inhibition at the early stage of viral life cycle was found. The viral morphology was found to be intact in the presence of the compound using transmission electron microscopy. Subsequent viral passages together with increasing compound concentrations gave rise to an escape mutant clone with a methionine to leucine substitution at amino acid residue 402 of the HA confirmed by whole genome sequencing. The escape mutant virus was resistant to high dosages of the compound and the resistance level of the mutant was further confirmed by the recombinant virus generated through reverse genetics. Molecular docking using available HA crystal structures suggests that the compound may bind favorably to a pocket on HA in which M402 is located. These results strongly suggest that the compound may inhibit viral entry by targeting HA via binding to M402. Available three-dimensional structure indicates that M402 is located on the B-loop of HA2 subunit which is involved in loop-to-helix transition during membrane fusion. As this transition is critical in the fusion process, we speculate the compound inhibits viral infection by targeting this step. To evaluate this hypothesis, we carried out trypsin protection assay and acid inactivation assay and subsequent experimental results from both assays support our postulations. This study may thus benefit the characterization and development of novel fusion inhibitors of influenza A viruses.

121 TSR-026 – An Oral Drug Therapy for Oseltamivir-Resistant Influenza Infections

John M. Hilfinger[1], Donald F. Smee[2], Dawn M. Reyna[1], Mindy A. Collins[1], Crystal A. Jurkiewicz[1], Elke Lipka[1]
[1]TSRL, Inc., Ann Arbor, USA, [2]Utah State University, Logan, USA

Zanamivir and oseltamivir carboxylate are potent inhibitors of influenza virus neuraminidase, a key enzyme in the life cycle of the virus. These neuraminidase inhibitors have potent virus-inhibitory activities in cell culture systems, yet as a class of compounds, neuraminidase inhibitors suffer from poor membrane, typically showing ~ 2% bioavailability in humans. TSRL has developed prodrugs of zanamivir (TSR-026) and a guanidino analog of oseltamivir (TSR-462) with increased oral bioavailability and demonstrated activity against a number of influenza virus strains including strains possessing the common neuraminidase gene mutation H275Y that confers resistance to oseltamivir carboxylate (OC). Compounds were tested in a mouse model of influenza. Female 16-18 g BALB/c mice were exposed intranasally with a lethal doses of Influenza A/NWS/33 (H1N1), A/California/04/2009 (H1N1), A/Victoria/3/75 (H3N2) and A/Duck/MN/1525/81 (H5N1) and A/Mississippi/3/2001 (H1N1) H275Y. Mice were treated orally with TSR-026 and TSR-462, controls or placebo for 5 days. Study endpoints were % survival, mean time to death, lung hemorrhage score, lung virus titer, and drug levels in plasma. TSR-026 and TSR-462 were generally as effective or superior to oseltamivir against the H1N1, H3N2 and H5N1 strains. In the oseltamivir-resistant strains, the order of protection from weight loss, disease severity and mortality was as follows: TSR-026 = Ribavirin >TSR-462 > oseltamivir > placebo. Treatment effectively reduced lung hemorrhage scores and lung weights. The results demonstrate that TSR compounds show broad efficacy against circulating influenza strains and are superior to oseltamivir in the treatment of an oseltamivir-resistant influenza A H1N1 virus infection in mice. TSR-026 shows great potential as an orally active, zanamivir-like inhibitor of oseltamivir-resistant viruses *in vivo*.
This work was sponsored by the NIAID Phase II SBIR 2 R44 AI081396-02 and Contract HHSN272201000039I/ HHSN27200008/A44 from the Respiratory Diseases Branch, DMID, NIAID, NIH.

122 A Ferret Model to Facilitate the Establishment of Laboratory Correlates for Clinically Relevant Oseltamivir Resistance

H Marjuki, AP Chesnokov, VP Mishin, K Sleeman, M Okomo-Adhiambo , AI Klimov, LV Gubareva
Influenza Division, NCIRD, CDC, Atlanta, USA

The neuraminidase (NA) inhibitor (NAI) oseltamivir is the most widely prescribed antiviral drug for the management of influenza A and B virus infections. To monitor susceptibility to the NAI class of drugs, surveillance laboratories worldwide utilize NA inhibition (NI) assay supplemented with genotypic analysis. Selected virus variants exhibiting "reduced" or "highly reduced" inhibition in the NI assay, as determined by WHO WER (28/09/2012), are used as reference viruses and included in the international isirv-AVG panel. However, whether oseltamivir treatment would produce an inhibitory effect on the variant virus' replication in patients remains unknown. Since humans and ferrets share a high degree of similarities in respiratory tract receptors and disease symptoms, ferrets provide a valuable animal model to study properties of influenza viruses. In this study, we established a ferret model and tested the reference influenza B virus variant carrying the D197E substitution in the NA, as well as its wild-type (WT) counterpart. When determined in the NI assay, the oseltamivir-IC_{50} value (63.1 nM) of the D197E variant was 8-fold greater (categorized as "reduced inhibition") compared to that of the WT virus (IC_{50}=7.6 nM). Our questions were i) whether the D197E substitution alters virus fitness; and ii) whether oseltamivir treatment inhibits replication of the D197E variant virus *in vivo*. The results showed a comparable fitness (judged by peak virus titers in the nasal washes, duration of shedding and symptoms) between WT and D197E variant viruses in ferrets. In treatment experiments, we observed a dose-dependent antiviral effect in WT-infected ferrets, whose viral titers were significantly reduced in the group treated with 2.5 mg/kg of oseltamivir twice daily for 5 days (equivalent human dosage) or with 12.5 mg/kg dose. In contrast, D197E virus titers were unaffected by 2.5 mg/kg oseltamivir treatment. Small, but statistically significant reduction in virus titers was found on day 2 post-challenge at the higher drug dose. Altogether, the data suggest that the D197E variant is likely to be resistant to oseltamivir in humans.

123 A Novel Class of Highly Potent Small Molecule Inhibitors of Entero/rhinovirus Replication with an Excellent Safety and Pharmacokinetic Profile are Highly Effective Against Enterovirus Infections in Mice

Hendrik Jan Thibaut[1], Sung-Hoon Ahn[2], Aloys Tijsma[1], Chong-Kyo Lee[2], Eric Verbeken[3], Young-Sik Jung[2], Johan Neyts[1]

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A series of highly potent and broad-spectrum inhibitors of entero- and rhinovirus replication was developed. We here describe the early preclinical profile and *in vivo* efficacy of the most potent congener of this series, KR-23247 (an analogue of KR-22809, Thibaut et al.). We demonstrated that this molecule displays a highly favorable *in vitro* and *in vivo* ADME-Tox profile (the following parameters were assessed: metabolic and plasma stability, cell permeability, effect on CYP450 activity, hERG binding, *in vivo* and *in vitro* toxicity and plasma protein binding). The *in vivo* efficacy of KR-23247 was determined in a relevant CVB4-induced pancreatitis model (the following parameters were assessed: histopathological scoring of pancreatitis severity, effect on serum amylase and lipase levels, infectious virus titers and viral RNA levels in the pancreas). CVB4-infected mice were completely protected in a dose-dependent manner from virus-induced pancreatitis when treated either subcutaneously or *per os* with KR-23247. Even once daily dosing of 20mg/kg given by the oral route, starting 36h post infection resulted in complete inhibition of virus-induced pancreatitis. KR-23247 also protected against CVB4-induced mortality in SCID-mice when given orally a dose of 20mg/kg once daily. Given the broad-spectrum anti-entero/rhinovirus activity, this class of molecules has the potential to be used for the treatment of (life-threatening) enteroviral infections as well as for prophylaxis and treatment of rhinovirus induced exacerbations of COPD and asthma. Supported by EU FP7 project SILVER (grant 260644)

ORAL SESSION 5: HERPESVIRUSES AND POXVIRUSES

Chairs: Rhonda Cardin, Ph.D. and Sophie Duraffour, Ph.D.

8:30 – 10:00 am

BALLROOM BC

181 The Reverse Transcriptase Inhibitor Tenofovir (TFV) Also Targets the Herpes Simplex Virus (HSV) DNA Polymerase

Graciela Andrei, Sarah Gillemot, Robert Snoeck

Rega Institute for Medical Research, KU Leuven, Belgium

HSV is a common cause of both genital and oral disease. HSV-2 is mostly a sexually transmitted pathogen while HSV-1 is frequently acquired during early childhood, mainly via oral secretions. However, the epidemiology of HSV-1 is changing, with increased frequency of sexual transmission of HSV-1. Genital herpes is an important cofactor for acquisition of HIV infection and effective prophylaxis is a helpful strategy to halt both HIV and HSV transmission. In the CAPRISA004 study, the antiretroviral agent TFV, formulated as a vaginal microbicide gel, was shown to reduce HIV acquisition by 39% in women with the additional finding that TFV gel reduced the risk of HSV-2 infection by 51%. We have shown that at the concentration achieved intravaginally with a 1% TFV topical gel, TFV had direct anti-herpetic activity. TFV affected HSV replication in several cell culture systems at relative high concentrations (EC_{50} in the range of 80-200 µg/ml) compared to reference anti-HSV drugs. The active TFV metabolite inhibited both HSV DNA polymerase and HIV reverse-transcriptase activities in enzymatic assays. Here, we report the phenotypic (drug-resistance profiling) and genotypic (sequencing of the DNA polymerase gene) characterization of HSV-1 (Kos strain) and HSV-2 (G strain) mutants selected for resistance to TFV by stepwise dose escalation of the drug. Several plaque-purified viruses isolated from 3 independent selection procedures with HSV-1 harbored the L802F amino acid substitution in the conserved region III of the catalytic domain of the viral

DNA polymerase. HSV-2 mutants isolated from two separated selection procedures had the A724V amino acid change in the conserved region II of the viral enzyme. These amino acid changes have been previously described in association with resistance to foscavir and acyclovir. The TFV-resistant HSV-1_{L802F} and HSV-2_{A724V} mutants showed cross-resistance with PME derivatives [adefovir (PMEA) and PMEDAP] but not with HPMP derivatives (cidofovir, HPMPA, and HPMP-5-azaC). Cross-resistance between TFV with PME derivatives, foscavir and acyclovir but not with HPMP derivatives was confirmed when several clinical and laboratory strains bearing well-characterized mutations in the viral DNA polymerase were evaluated.

182 Selection and Recombinant Phenotyping of a Novel CMX001 and Cidofovir Resistance Mutation in Human Cytomegalovirus

Mark Prichard[1], Nathan Price[1], Caroll Hartiline[1], Randall Lanier[2], Scott James[1]
[1]*University of Alabama at Birmingham, Birmingham, USA,* [2]*Chimerix Inc., Durham, USA*

CMX001 is an orally available lipid acyclic nucleoside phosphonate that delivers high intracellular levels of cidofovir (CDV)-diphosphate and exhibits enhanced *in vitro* antiviral activity against a wide range of double-stranded DNA viruses, including cytomegalovirus (CMV). Mutations in the DNA polymerase of CMV that impart resistance to CDV also render the virus resistant to CMX001. Here, we report a novel resistance mutation that arose under the selective pressure of CMX001. The wild-type CMV strain AD169 was propagated in human foreskin fibroblasts under increasing concentrations of CMX001 over 10 months and the resulting strain ($CMX001^R$) was less susceptible to CDV and CMX001 in a plaque reduction assay. Genotypic analysis of $CMX001^R$ via conventional sequencing of the genes encoding the CMV DNA polymerase (*UL54*) and UL97 kinase (*UL97*) demonstrated one mutation which changed the wild-type aspartate to glutamate at position 542 in *UL54*. A recombinant virus with this novel D542E mutation was generated via bacterial artificial chromosome-mediated marker transfer experiments. Subsequent phenotypic resistance analysis of the D542E mutant demonstrated reductions in susceptibility of greater than 10-fold to CMX001 and CDV but no resistance to foscarnet (FOS) or ganciclovir (GCV). Analysis of replicative fitness showed that both $CMX001^R$ and the D542E mutant viruses demonstrated a smaller plaque phenotype and slower replication kinetics than their respective parent viruses. These data describe the first resistance mutation generated under the selective pressure of CMX001 and suggest that CMX001 may have a unique resistance profile associated with reduced viral replication and maintenance of sensitivity to FOS and GCV.

ACKNOWLEDGMENTS: These studies were funded in whole or in part with federal funds from National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contracts N01-AI-30049 and HHSN27220110000010C

183 Efficacy of Tranylcypromine (TCP) against Herpes Simplex Virus Type 2 in Murine and Guinea Pig Models

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Chromatin complexes which regulate herpes simplex virus (HSV) gene expression during lytic and latency reactivation represent novel antiviral targets. Tranylcypromine (TCP), a monoamine oxidase inhibitor (MAOI), inhibits histone H3-Lysine 9 demethylase LSD1 (lysine-specific demethylase-1), an enzyme required for initiation of HSV immediate-early gene expression. To determine whether TCP inhibits HSV infection *in vivo*, mice were pre-treated orally twice daily with TCP (7.5 mg/kg), ACV (50 mg/kg) or vehicle for 7 days and inoculated intranasally with HSV-2, strain MS. At 10 days post infection (dpi), viral DNA loads were significantly reduced in peripheral tissues such as the lung and spleen, and in neural tissues such as the cerebral cortex, cerebellum, and trigeminal ganglia by both treatments. Continuous pellet delivery of 5, 2.5, or 1.25 mg of TCP to HSV-2 infected mice also exhibited a dose-dependent reduction of HSV-2 in ganglia. Finally, i.p. administration of TCP (5 and 10 mg/kg) to mice following ocular HSV-1 inoculation significantly reduced viral loads in ganglia. To determine whether TCP

treatment reduces recurrent lesions and recurrent vaginal shedding, TCP was administered to HSV-2 infected guinea pigs i.p. twice daily beginning at 15 days post vaginal HSV-2 inoculation and continued for 21 days at 15 mg/kg/day and compared to ACV treatment or vehicle. Cumulative recurrent lesion scores (days 15-63) were significantly reduced to 5.4 in TCP-treated animals (p=0.04) and to 3.6 in ACV-treated animals (p=0.004) versus 9.1 in vehicle-treated animals. Recurrent vaginal shedding in the TCP-treated group was also significantly reduced during the treatment phase (p=0.04). A higher treatment dose of TCP (30 mg/kg/day) also reduced recurrent disease. Significantly, we demonstrated that TCP effectively reduces infection in neural tissues, reactivation and recurrent disease. Thus, LSD1 inhibitors warrant further investigation as anti-HSV treatments [supported by NIAID, NIH contracts HHSN272201000008I (CCHMC) and HHSN272201000007I (UAB)]

184 KAY-2–41: A Novel Nucleoside Analogue Inhibitor of Orthopoxviruses

Sophie Duraffour[1], Kazuhiro Haraguchi[2], Jan Balzarini[1], Kaori Yamada[2], Hirochimi Tanaka[2], Joost J van den Oord[3], Graciela Andrei[1], Robert Snoeck[1]

[1]Rega Institute for Medical Research, KU Leuven, Belgium, [2]Showa University, School of Pharmacy, Japan, [3]University Hospitals Leuven, Laboratory of Translational Cell and Tissue Research, Belgium

In the current context of increasing *Orthopoxvirus* (OPV) infections, the access to adequate treatments appears valuable not only for human, but also for veterinary use. In the search for novel antiviral agents, a 1' -carbon-substituted 4 -thiothymidine derivative, i.e. KAY-2-41 [1-(2-deoxy-1-methyl-4-thio-β-D-ribofuranosyl)-thymine], emerged as a potent inhibitor of poxviruses, and we describe here its *in vitro* and *in vivo* antiviral effects. In cells, KAY-2-41 was active in the nanomolar range against various OPVs and in the micromolar range against the parapoxvirus orf. The compound preserved its antiviral potency against vaccinia (VACV) and camelpox (CMLV) strains resistant to cidofovir and/or (*S*)-HPMPDAP. Mutations in both exonuclease and polymerase domains of E9L (A314V+A684V) appeared to enhance KAY-2-41 activity. The molecule had no noticeable toxicity on confluent cell cultures, but cytostatic effect was seen on growing cells. Genotyping of VACV, cowpox virus (CPXV) and CMLV selected for resistance to KAY-2-41 led to the identification of mutations in the thymidine kinase (*J2R*) but not in the thymidilate kinase (*A48R*) nor in the viral DNA polymerase (*E9L*) genes. Such viruses depicted relatively low levels of resistance to KAY-2-41 ranging from a 2.7- to 6-fold change in EC_{50} values. Although thymidine kinase was assumed to be required for the phosphorylation of KAY-2-41, the compound was weakly phosphorylated by human thymidine kinases and not by VACV thymidine kinase. In mice, KAY-2-41 protected animals from mortality after a lethal challenge with VACV, reduced viral loads in the sera and suppressed virus replication to undetectable levels in lungs, liver, spleen and kidneys, without apparent toxicity. In conclusion, KAY-2-41 is a promising nucleoside analogue for the treatment of poxvirus-related infections. Its precise mode of action is currently under investigation.

185 Effects of Low and High Volume Intranasal Treatments with an Adenovirus Vectored Interferon (mDEF201) on Respiratory and Systemic Infections in Mice Caused by Cowpox and Vaccinia Viruses

Donald F. Smee[1], Min-Hui Wong[1], Brett L. Hurst[1], Jane Ennis[2], Jeffrey D. Turner[2]

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An adenovirus 5 vector encoding for mouse interferon alpha, subtype 5 (mDEF201), was evaluated for efficacy against lethal cowpox (Brighton strain) and vaccinia (WR strain) virus respiratory and systemic infections in mice using low (5-µl) and high (50-µl) intranasal treatment volumes. The purpose was to compare differences in efficacy between the two volumes, since treatment of humans would be in a small volume that would stay confined to the upper respiratory tract. Lower respiratory infections (LRI), upper respiratory infections (URI), and systemic infections were induced by 50-µl intranasal, 10-µl intranasal, and 100-µl intraperitoneal infection volumes of virus, respectively. Most mDEF201 treatments were given prophylactically 24 hours prior to virus challenge. Single treatments of 10^6 and 10^7 PFU/mouse of mDEF2021 in a 5-µl volume protected all mice from vaccinia-induced LRI mortality (comparable to prior published studies with 50-µl treatments). Systemic vaccinia infections responded better to 5-µl mDEF201 treatments than to 50-µl treatments. Cowpox LRI infections responded to 10^7 mDEF201

treatments, but a 10^6 dose was only weakly protective. Cowpox URI infections were approximately equally treatable by 5- and 50-µl volumes of mDEF201 at 10^7PFU/mouse. Dose-responsive prophylaxis with mDEF201 given one time only 56 days prior to initiating a vaccinia virus LRI infection was 100% protective from 10^5 to 10^7 PFU/mouse. Improvements in lung hemorrhage score and lung weight were evident, as were decreases in virus titers in livers, lungs, and spleens. Thus, mDEF201 was able to treat different vaccinia and cowpox virus infections using both small and large treatment volume regimens. Supported by Contract No. HHSN272201000039I/HHSN2720006/A30 from the Virology Branch, DMID, NIAID, NIH.

186 Feline Herpesvirus Ocular Disease – Developing an Antiviral Ophthalmic Solution for Cats

Nesya Goris[1], Joeri Auwerx[1], Eleonóra Kiss[1], Jérôme Villers[1], Aino Billiet[1], Philippe Hansen[2], Johan Neyts[1,3]
[1]Okapi Sciences NV, Heverlee, Belgium, [2]ECVO Diplomate , Brussels, Belgium, [3]Rega Institute (KU Leuven), Leuven, Belgium

Feline herpesvirus 1 (FHV-1), a member of the *Alphaherpesvirinae*, is one of the most common viral pathogens of domestic cats and the causative agent of feline viral rhinotracheitis. In addition, FHV-1 has a predilection for corneal epithelium resulting in an array of ocular disease manifestations and on occasion even blindness in cats.

Although antiviral therapy has become standard practice in the management of human herpesvirus infections, no specific anti-herpes drug has been licensed for veterinary application. Here we report on the pre-clinical and clinical development of a nucleoside analogue into a specific ophthalmic solution for the treatment of FHV-1 ocular disease in cats. The active substance displayed an *in vitro* anti-FHV-1 activity superior to aciclovir, ganciclovir, penciclovir and cidofovir. This nucleoside analogue is activated by the viral thymidine kinase and the 5'-triphosphate is a selective inhibitor of the herpesvirus polymerase. A series of local tolerance, pharmacokinetic and dose-determination and dose-confirmation studies led to the selection of a safe, well-tolerated and effective formulation [Figure: six-week old European shorthair male with signs of FHV-1 induced conjunctivitis and dendritic ulceration before treatment (left) and following 7 days of treatment (right) with the ophthalmic solution]. The established dosing regimen of 0.2% w/v ophthalmic solution administered three times daily by topical instillation of a single drop per eye per application is currently being confirmed in a pivotal randomized, placebo-controlled, blinded, multi-centric in-use safety and effectiveness field trial in cats with naturally occurring FHV-1 ocular disease.

ORAL SESSION 6: EMERGING INFECTIONS

Chairs: Mike Bray, M.D. and Justin Julander, Ph.D.

10:30 – 12:30 pm

BALLROOM BC

187 Expecting the Unexpected: Targeting Bat Coronaviruses in Preparation for Their Human Descendants

Rolf Hilgenfeld[1,2,3], Yibei Xiao[1,2], Shyla George[1,2], Daizong Lin[3], Hong Liu[3], Christian Drosten[4], Yuri Kusov[1,2], Qingjun Ma[1,2]
[1]Institute of Biochemistry, University of Lübeck, Lübeck, Germany, [2]German Center for Infection Research, University of Lübeck, Lübeck, Germany, [3]Shanghai Institute of Materia Medica, Shanghai, China, [4]University of Bonn Medical Center, Bonn, Germany

Very recently, a new human coronavirus was reported that led to severe respiratory disease in a limited number of cases (1). Provisionally designated HCoV EMC, this virus belongs to Betacoronavirus subgroup c and is very similar to the coronaviruses HKU4 and HKU5 that had been isolated from bats (2). We have determined crystal structures of the main proteases (Mpros) of several bat coronaviruses in an effort to contribute to preparedness against zoonotic transmission of these viruses into the human population. The three-dimensional structures of the

Mpros of the bat-CoVs HKU4 and HKU8 as well as of the new human coronavirus EMC will be discussed. The enzymes from HKU4 and HCoV EMC feature significant differences from the main protease of SARS coronavirus (3). We have also found that our broad-spectrum enterovirus inhibitor, SG85 (4), is highly active against the main protease of HKU4. Tests with EMC-infected cell cultures revealed potent antiviral activity of SG85. Further, we designed alpha-ketoamide inhibitors for the main protease of HKU4 and demonstrated good anti-HCoV EMC activity for these as well. Both SG85 and the alpha-ketoamides are non-toxic in several cell types (CC_{50} = 265

188 Novel Inhibitors of SARS-CoV Entry Acting by Three Distinct Mechanisms

Adeyemi O. Adedeji[1,3], William Severson[5], Colleen Jonsson[6], Susan R. Weiss[4], Stefan G. Sarafianos[1,2]
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Severe acute respiratory syndrome (SARS) is an infectious and highly contagious disease that is caused by SARS-associated coronavirus, (SARS-CoV) and for which there are currently no approved treatments. We report the discovery and characterization of small molecule inhibitors of SARS-CoV replication that block viral entry by three different mechanisms. The compounds were discovered by screening a chemical library of compounds for blocking entry of HIV-1 pseudotyped with SARS-CoV surface glycoprotein S (SARS-S), but not with Vesicular Stomatitis Virus surface glycoprotein G (VSV-G). Studies on their mechanism of action revealed that they act by three distinct mechanisms: a) SSAA09E2 (N-[[4-(4-methylpiperazin-1-yl)phenyl]methyl]-1,2-oxazole-5-carboxamide) acts through a novel mechanism of action, by blocking interactions of SARS-S with the receptor for SARS-CoV, Angiotensin Converting Enzyme-2 (ACE2). b) SSAA09E1 ([(Z)-1-thiophen-2-ylethylideneamino] thiourea) blocks cathepsin L, a host protease required for processing of SARS-S during viral entry. c) SSAA09E3 (N-(9,10-dioxo-9,10-dihydroanthracen-2-yl)benzamide)), does not affect interactions of SARS-S with ACE2 or the enzymatic functions of cathepsin L , but prevents fusion of the viral membrane with the host cellular membrane. Inhibitors from all three classes were found to have potent antiviral activity against infectious SARS-CoV. Our work demonstrates that there are at least three independent strategies to block SARS-CoV entry, validates novel mechanisms of inhibition, and introduces promising leads for the development of SARS therapeutics.

189 A Novel Class of Host Mediated Antiviral Drugs Demonstrate Potent Inhibition of Dengue Virus Type 2

Ikenna M. Madu[1], Shari Kaiser[1], Myra Wang[1], Kerry Fowler[1], Sowmya Pattabhi[2], Micheal Gale Jr.[2], Shawn P. Iodonato[1], Kristin M. Bedard[1]
[1]Kineta Inc., Seattle, USA, [2]University of Washington, Seattle

We have identified a novel class of small molecule antiviral drugs that are effective against the arthropod-borne Dengue virus (DENV) through host mediated antiviral mechanisms. DENV is a pathogen of global health concern causing manifestations ranging from dengue fever which is flu-like to the dengue hemorrhagic fever/dengue shock syndrome. In the absence of effective vaccines against DENV alternative treatments are in high demand. We have identified small molecule drugs that trigger a natural immune response by targeting the retinoic acid inducible gene I protein (RIG-I) a critical first responder found in most cells in the body that is critical to suppressing viral replication and clearing infection. We describe a class of benzothiazole compounds that stimulate IRF-3 translocation and other downstream effectors of RIG-I and cause a potent inhibition of DENV-2 viral replication in various cell types *in vitro* including Huh7, 293 and THP-1 cells. The activity of these compounds has been shown dependent on the RIG-I pathway as cell lines lacking IRF-3 expression are not responsive to immune stimulation following drug treatment. The compounds have been optimized through medicinal chemistry and are effective at nanomolar concentrations with a therapeutic index of greater than 20 in cell based antiviral assays. These novel antiviral compounds maintain potent antiviral activity when added after dengue virus type 2 (DENV-2) infection

is established supporting their development as novel antiviral treatments. Furthermore, the compounds are well tolerated *in vivo* with a maximum tolerated ≥10 mg/kg in Sprague Dawley rat, with no adverse events or clinical symptoms observed. Ongoing studies are focused around developing an oral formulation of the benzothiazole antivirals and defining their activity utilizing animal models of DENV infection.

190 A Novel, Convenient and Robust Reverse Genetic System for the Production of a DNA-based Live Attenuated Vaccine Against the Yellow Fever Virus

K. Dallmeier, J. Neyts
Rega Institute for Medical Research, KU Leuven, Belgium

The yellow fever virus (YFV), causes severe and life-threatening infections with jaundice, systemic bleeding, shock and multi-organ failure. A combined population of over 900 million people in Africa and Latin America is at risk. A safe and highly efficient live attenuated prophylactic vaccine is available [YFV-17D, Stamaril ®] that induces effective immunity within one week for 95% of persons vaccinated. Despite the availability of this vaccine, an estimated 200.000 cases of yellow fever occur each year resulting in ~30.000 deaths. Vaccination with Stamaril ®, [which was developed more than 80 years ago] is recommended for people living in and travelling to high risk regions. Prompt detection of yellow fever and rapid response through emergency vaccination campaigns is essential to control outbreaks (often in remote and resource poor areas). The need for a proper cold-chain complicates however the timely delivery of vaccines. An easy and inexpensive to produce DNA vaccine would allow much faster and simplified deployment of mass vaccination efforts. We developed a unique and novel reverse genetic system, using an easily tractable multi-host shuttle vector system, for the convenient launching of recombinant flavivirus genomes. This system allows to bypass almost all the problems currently encountered during cloning, maintenance and manipulation of highly unstable (toxic) viral cDNAs. The system allows to produce clonal viruses by direct launching from easily scalable plasmid DNAs. We demonstrate that the particular characteristics of the YFV-17D thus generated are identical to that of the original vaccine virus (such as efficiency of replication, virus yield and plaque phenotype). Moreover, when this naked YFV-17D plasmid DNA was injected (i.p.) in AG129 mice, it resulted in the same pathology, morbidity and mortality as the parent virus. This convenient, robust and reproducible system may allow to develop a DNA vaccine for YFV at low costs without the need for eukaryotic cell cultures or embryonated chicken eggs. It will no longer require a cold-chain and might be administered needle free.

191 Structure of Norovirus RNA-dependent RNA-polymerase in Complex with Three Naphthalene Derivates Inhibitors

Eloise Mastrangelo[1,2], Delia Tarantino[2], Romina Croci[2], Margherita Pezzullo[1,2], Martino Bolognesi[1,2], Mario Milani[1,2]
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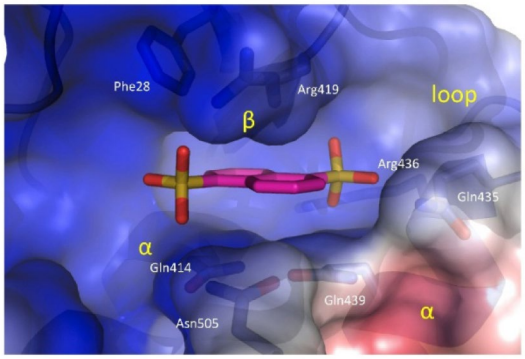
Noroviruses (NV) are members of the Calicivirus family of positive sense RNA viruses. Nowadays noroviral gastroenteritis is responsible for about 200,000 deaths a year in developing countries. Despite the obvious medical relevance of NV infections, still no effective vaccines/ antiviral treatments are available. Following the identification of Suramin and NF023 as NV RNA-dependent RNA-polymerase (RdRp) inhibitors (Mastrangelo et al., J. Mol. Biol. 2012. 419:198-210) to better characterize the inhibition mechanism we started the analyses of what we consider the active 'heads' of these two molecules i.e. naphtalene di- and tri-sulfonates (NAF2 and NAF3, respectively) and of a related molecule: Pyridoxal-5'-phosphate-6-(2'-naphthylazo-6'-nitro-4',8'-disulfonate) tetrasodium salt (PPNDS). The 3 compounds have been tested in enzymatic RdRp inhibition assays, displaying medium to high activity against human- and murine-NV



h-RdRp/NAF2 complex: the high affinity site

RdRps (h-RdRp and M-RdRp, respectively). We solved the crystal structures of M-RdRp with the three inhibitors and of h-RdRp with NAF2 showing an unexpected additional binding site besides that observed in NF023/Suramin complexes. The new site is located in the RdRp 'thumb' domain in a cleft along the exit pathway of the newly formed RNA (Fig. 1). As evidenced by crystallographic data, NAF molecules have higher affinity for the new binding site (accordingly named high affinity site). In fact, only when the concentration of the NAFs is increased, also the old inhibition site is occupied. We discuss the different properties of the new inhibition site in NV-RdRps.

192 The Nucleoside Analogue 2'-C-methylcytidine Inhibits Norovirus Replication *in vitro* and Protects Against Virus Induced Diarrhea and Mortality in a Mouse Model

Joana Rocha-Pereira[1], MSJ Nascimento[1], Johan Neyts[2], Dirk Jochmans[2]
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Noroviruses are the most common cause of outbreaks of foodborne illness and are responsible for the death of ~200 000 children in developing countries. There is no vaccine or antiviral drug available. We reported recently that the HCV nucleoside polymerase inhibitor 2'-*C*-methylcytidine (2CMC) inhibits the *in vitro* replication of the murine norovirus (MNV) (Rocha-Pereira et al., 2012). We now demonstrate that 2CMC also inhibits the replication of the Norwalk virus replicon. 2CMC was able to clear cells from their replicon following four consecutive passages of antiviral pressure. The generation of MNV 2CMC-resistant variants proved unsuccessful even following 30 weeks of culturing in suboptimal concentrations. This indicates that the barrier to resistance, as is the case for HCV, is high. To evaluate the potential *in vivo* anti-norovirus effect of 2CMC, we established a MNV-infection model in AG129 mice (129/Sv mice deficient in both α/β and γ interferon receptors). Whereas untreated infected mice developed severe diarrhea, lost 14 $\pm$ 1 % body weight by day 3 post-infection (pi) and died with a mean day of death of 4,2 $\pm$ 0,4 days, all mice that were treated with 2CMC (2 x 50 mg/kg/day for 7 consecutive days) survived the infection without developing diarrhea. Treated mice had lost < 10% of body weight by day 5 pi. They shed virus in the stool at day 5 and 6 pi but appeared otherwise healthy and regained body weight as of day 6 pi. Following cessation of 2CMC treatment, the mice were kept for 21 days and remained in good health during the entire period of time. We here demonstrate, to the best of our knowledge, for the first time that a small molecule inhibitor of norovirus replication protects, in a relevant animal model, against norovirus induced diarrhea and mortality. These findings open perspectives for the development of potent and safe norovirus inhibitors to treat and prevent (including during outbreaks) norovirus infections. Supported by EU FP7 project SILVER (grant 260644).

193 Identification of Acute Respiratory Failure and Long-term Motor Function Deficits as Therapeutic Targets for Arboviral Encephalitides

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Utah State University, Logan, USA

Respiratory distress and motor function deficits are recognized as serious outcomes for arboviral encephalitis in human patients. Using rodent models, we have determined that damaged motor neurons in the phrenic spinal cord and respiratory control neurons in the medulla contribute to these neurological deficits of arboviral encephalitides. To explore the possibility that respiratory insufficiency is a broad mechanism of death of viral encephalitis, whole body plethysmography was evaluated in mice infected with three flaviviruses: WNV, Japanese encephalitis virus (JEV), North American tick-borne encephalitis Powassan virus (POWV), and two alphaviruses: neuroadapted Sindbis virus (NSV), and western equine encephalitis virus (WEEV). The death of mice infected with 4 of 5 of these viruses was strongly associated with the development of suppressed minute volume (MV). Conversely, mortality of mice infected with WEEV was not associated with suppressed MV. Neurological responses to hypercapnia (7% CO2) and optogenetic photoactivation of phrenic neurons in the cervical cord were dramatically diminished in mice challenged with WNV, NSV, and POWV, but not with WEEV. Moreover, optogenetic photoactivation of the C4 cervical cord did not stimulate diaphragmatic EMGs of WNV-, POWV-, and NSV-infected mice with very low MV. Neurons with the orexin-1 receptor protein in the ventral C3-5 cervical cord were statistically diminished

in WNV-infected mice with low MV as compared to WNV-infected or sham-infected mice without respiratory insufficiency. Moreover, long-term motor function deficits can be longitudinally measured in mice by optogenetic photoactivation of the lumbosacral spinal cord motor neurons for evaluation of therapies on long-term neurological sequelae. Identification of these common mechanisms of neuropathogenesis opens opportunities to "repurpose" drugs used for other indications with disease mechanisms similar to those occurring in arboviral encephalitides. Support: NIAID U54 AI-065357, NIAID/NIH HHSN272201000039I, and Utah Agriculture Research Station UTA00424

194 Prevention of Lethal Rift Valley Fever Virus Disease by Post-Exposure Administration of MP-12 Vaccine Derivatives Lacking NSs

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Rift Valley fever virus (RVFV) causes severe disease in humans and livestock. There are currently no approved antivirals or vaccines for the treatment or prevention of RVF disease in humans. A major virulence factor of RVFV is the NSs protein, which inhibits host transcription including the interferon-β gene and promotes the degradation of dsRNA-dependent protein kinase, PKR. We analyzed the efficacy of the live-attenuated MP-12 vaccine strain and MP-12 variants that lack the NSs protein as post-exposure vaccinations. Although parental MP-12 failed to elicit a protective effect in mice challenged with wild-type (wt) RVFV by the intranasal route, significant protection was demonstrated by vaccination with MP-12 strains lacking NSs when administered subcutaneously (s.c.) at 20 to 30 min post-exposure. Viremia and virus replication in liver, spleen and brain were also inhibited by post-exposure vaccination with MP-12 lacking NSs. The protective effect was substantially reduced when vaccination was delayed until 6 h after intranasal RVFV challenge. When mice were challenged s.c., efficacy of contralaterally administered MP-12 lacking NSs was diminished, most likely due to more rapid dissemination of wt RVFV. Our findings suggest that post-exposure vaccination with MP-12 lacking NSs may be developed as a novel post-exposure treatment to prevent RVF. Acknowledgment: Supported by contract HHSN272201000039I from the NIAID, NIH.

ORAL SESSION 8: CLINICAL SYMPOSIUM

Chairs: Joseph Colacino, Ph.D. and Randall Lanier, Ph.D.

1:00 – 3:30 pm

BALLROOM BC

204 STaR Study: Single Tablet Regimen Rilpivirine/Emtricitabine/Tenofovir DF Is Non-Inferior to Efavirenz/Emtricitabine/Tenofovir DF in ART-Naïve Adults

Calvin Cohen[1], David Wohl[2], Jose Arribas[3], Keith Henry[4], Hui Wang[5], Danielle Porter[5], Shampa De-Oertel[5], Damian McColl[5]

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Simplified ARV regimens improve patient quality of life and long-term medication adherence. Rilpivirine/Emtricitabine/Tenofovir DF (RPV/FTC/TDF) and Efavirenz/Emtricitabine/Tenofovir DF (EFV/FTC/TDF) are

once-daily, single-tablet regimen (STR) HIV treatment options. This is the first study to directly compare safety and efficacy of the two STRs, RPV/FTC/TDF and EFV/FTC/TDF, in treatment-naïve adults. STaR is an ongoing, open-label, international, 96-week study to evaluate the safety and efficacy of the STR RPV/FTC/TDF compared to the STR EFV/FTC/TDF in treatment-naïve HIV-1 infected subjects. Subjects were randomized 1:1 to RPV/FTC/TDF or EFV/FTC/TDF. Eligibility criteria included screening HIV-1 RNA ≥ 2,500 c/mL, genotypic sensitivity to EFV, FTC, TDF, and RPV, and no prior ARV therapy. Randomization was stratified by HIV-1 RNA level (≤100,000 c/mL or >100,000 c/mL) at screening. The primary endpoint was the proportion of subjects with HIV-1 RNA <50 c/mL at W48 as determined by FDA snapshot algorithm (12% non-inferiority margin). A total of 786 subjects were randomized and received at least 1 dose of study drug (RPV/FTC/TDF, n=394; EFV/FTC/TDF, n=392). Baseline (BL) characteristics were similar between arms, with a BL mean CD4 count of 391 cells/mm^3 and HIV-1 RNA of 4.8 log10 c/mL. RPV/FTC/TDF was non-inferior to EFV/FTC/TDF (86% vs 82%) at W48 for HIV RNA <50 c/mL (difference 4.1%, 95% CI [-1.1%, 9.2%]) per FDA snapshot analysis. A statistically significant difference was demonstrated for BL HIV-1 RNA ≤100,000 c/mL (n=510), 89% RPV/FTC/TDF vs 82% EFV/FTC/TDF (difference 7.2%, 95% CI [1.1%, 13.4%]), and non-inferiority for >100,000 c/mL (n=276), 80% RPV/FTC/TDF vs 82% EFV/FTC/TDF (difference -1.8%, 95% CI [-11.1%, 7.5%]). Overall, virologic failure was 8% for RPV/FTC/TDF vs 6% for EFV/FTC/TDF (difference 2.7%, 95% CI [-0.9%, 6.2%]). There were fewer study drug discontinuations due to AEs by FDA snapshot analysis in RPV/FTC/TDF (2%) compared to EFV/FTC/TDF (8%). The STR RPV/FTC/TDF demonstrated overall non-inferior efficacy and improved tolerability compared to the STR EFV/FTC/TDF.

205 CMV Resistance Profile of CMX001

Randall Lanier[1], Scott Foster[1], Sunwen Chou[2], Mark Prichard[3], Scott James[3], Tom Brundage[1], Herve Mommeja-Marin[1], Michelle Berrey[1]

[1]Chimerix Inc, Durham, USA, [2]Oregon Health and Science University, Portland, USA, [3]University of Alabama, Birmingham, USA

CMX001 is an orally administered lipid acyclic nucleoside phosphonate which is converted inside cells to cidofovir diphosphate (CDV-PP). CDV-PP acts as an alternative substrate inhibitor for the CMV viral DNA polymerase (UL54), the primary target for anti-CMV drugs including ganciclovir (GCV), CDV, and foscarnet (FOS). We report data here on the resistance profile of CMX001 from *in vitro* studies and two clinical trials: CMX001-201enrolled primarily antiviral naïve subjects (ClinTrials.gov NCT00942305) and CMX001-350 enrolled therapy experienced patients with life-threatening or serious diseases/conditions caused by one or more dsDNA viruses (ClinTrials.gov NCT01143181). *In vitro* passaging of wild type CMV with CMX001 selected a novel UL54 mutation (D542E) that conferred resistance to CMX001 and CDV, but not to GCV and FOS. The D542E mutation was associated with diminished viral fitness *in vitro*. In 201, no known resistance associated mutations (RAMs) were detected (N=171), but a polymorphism (R1052C) was detected in 3 patients. Phenotyping of R1052C demonstrated sensitivity to CMX001, CDV, GCV and FOS. In contrast to the absence of RAMs in this prevention study, many subjects in 350 had RAMs in either UL97 or UL54, consistent with known prior CMV antiviral use in the context of ongoing viral replication. CMV genotypes obtained for subjects with at least 4 weeks of CMX001 and detectable CMV at baseline (N=63) revealed UL54 predicted CDV resistance (CDV^{res}) at baseline or on therapy for 14 subjects while 21 subjects had UL97 mutations (GCV^{res}). As expected, UL97 RAMs had no discernible effect on response to CMX001. However, CDV^{res} UL54 RAMs were associated with a diminished response to CMX001. For example, at the last time on therapy, patients without CDV RAMs had a -0.81 (mean)/-1.0 (median) log10 decrease in CMV viremia (26/49 <200 copies/mL) while those with CDV RAMs had a -0.43 (mean)/-0.25 (median) log10 response (0/14 <200 c/mL). These data demonstrate that prior use of current CMV antivirals can compromise subsequent use of CMX001 when leading to UL54, but not UL97, RAMs and support that first line use of CMX001 could preserve subsequent therapeutic options.

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27th International Conference on Antiviral Research (ICAR)

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The 27th International Conference on Antiviral Research (ICAR), hosted by the International Society for Antiviral Research (ISAR), will take place at the Raleigh Convention Center, Raleigh, NC, USA. The conference will begin on Monday, May 12th, 2014, and will conclude on Thursday, May 15th, 2014.

ICAR provides an interdisciplinary forum of interest to chemists, biologists, and clinicians involved in antiviral research. At ICAR, scientists worldwide working in the areas of basic, applied, and clinical research meet in a collaborative and collegial atmosphere to review recent developments in all areas of antiviral drug discovery and development. Specific topics to be covered in the scientific program include: medicinal chemistry, virus replication, host cell-virus interactions, virus latency, new target identification, biochemistry and mechanism of action, mechanisms of viral drug resistance, assay development, in vitro evaluation, animal models, pharmacokinetics, toxicology, and clinical trials. Within these areas of interest, there will be keynote, plenary, and overview speakers, single topic minisymposia, a clinical symposium, and oral and poster presentations.

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