



28th
International Conference
on Antiviral Research
ICAR



Hosted by

ISAR

INTERNATIONAL SOCIETY
FOR ANTIVIRAL RESEARCH

Program and Abstracts



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DAILY SCHEDULE

MONDAY, MAY 11, 2015

- › Women in Science Roundtable
- › Drug Development 101 – Microbicide/PRP
- › Welcome and Keynote Address
- › Opening Reception

TUESDAY, MAY 12, 2015

- › RNA Viruses Symposium
- › Lunch (on your own)
- › Gertrude Elion Award Lecture
- › Oral Sessions – Drug Screening Technologies
- › Oral Sessions – In Vitro Evaluation and Antiviral Resistance
- › Poster Session I
- › New Member and First Time Attendee Networking Event

WEDNESDAY, MAY 13, 2015

- › Antonin Holý Award Lecture
- › Oral Sessions – Antiviral Chemistry
- › Shotgun Presentation I
- › Career Networking and Discussion Luncheon
- › Lunch (on your own)

THURSDAY, MAY 14, 2015

- › William Prusoff Young Investigator Award Lecture
- › Emerging Viruses Symposium
- › Lunch (on your own)
- › Oral Sessions – Targets and Mechanisms of Action
- › Shotgun Presentations II
- › Poster Session II
- › Closing Reception/Banquet

FRIDAY, MAY 15, 2015

- › HCV Plenary – Clinical Evaluation of Antiviral Therapies Animal Models of Infection



**International Society for Antiviral Research and
Twenty-Eighth International Conference on Antiviral Research**

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Frederick, MD, USA – 2018

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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 eighth year of existence, and has approximately 550 members representing 30 countries. Membership application forms will also be available at the Conference Registration desk, or from our website www.isar-icar.com.



CONTRIBUTORS

TO THE
28th International Conference
on Antiviral Research

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NETWORKING

KEYNOTE ADDRESS

MAY 11

**From the Elucidation of the HCV Life Cycle
to the Development of Highly Effective Antivirals**

Raffaele De Francesco, Ph.D.

Istituto Nazionale Genetica Molecolare, Milano, Italy
Doctors Without Borders Perspective on Ebola.

Armand Sprecher, M.D. M.P.H.

Doctors Without Borders, Brussels, Belgium

MAY 15

Advances in HCV Therapies

Michael Manns, M.D.

NETWORKING EVENTS

Women in Science Roundtable*

Monday, May 11, 2015
11:30 AM – 2:00 PM
LOCATION: ORSINI

Opening Reception

Monday, May 11, 2015
6:30 PM – 8:30 PM
LOCATION: FARNESE AND ORSINI

New Member and First Time Attendee Networking Event

Tuesday, May 12, 2015
6:45 PM – 7:30 PM
LOCATION: ORSINI

Career Networking and Discussion Luncheon*

Wednesday, May 13, 2015
12:00 PM – 1:30 PM
LOCATION: ORSINI

Conference Banquet

Thursday, May 14, 2015
Reception 7:30 PM
Dinner 8:00 PM – 10:00 PM
LOCATION: FARNESE AND ORSINI

**Space is limited and pre-registration is required.*



SCHEDULE at a GLANCE

MONDAY, MAY 11, 2015

TIME	EVENT	LOCATION
11:00 AM – 5:30 PM	Registration	REGISTRATION FOYER
11:30 AM – 2:00 PM	Women In Science Roundtable	ORSINI
2:00 PM – 4:00 PM	Drug Development 101	ESTENSI – MEDICI
4:45 PM – 6:15 PM	Keynote Addresses	ESTENSI – MEDICI
6:30 PM – 8:30 PM	Opening Reception <i>Light hors d'oeuvres served</i>	FARNESE AND ORSINI

TUESDAY, MAY 12, 2015

TIME	EVENT	LOCATION
7:00 AM – 5:30 PM	Registration	REGISTRATION FOYER
8:30 AM – 12:00 PM	RNA Viruses Symposium	ESTENSI – MEDICI
12:00 PM – 1:30 PM	Lunch	On Your Own
1:30 PM – 2:30 PM	Gertrude Elion Award Lecture	ESTENSI – MEDICI
2:30 PM – 4:30 PM	Oral Sessions – Drug Screening Techniques and In Vitro Evaluation and Antiviral Resistance	ESTENSI – MEDICI
4:30 PM – 6:30 PM	Poster Session I <i>Light hors d'oeuvres served</i>	FERNANDES
6:45 PM – 7:30 PM	New Member and First Time Attendee Networking Reception <i>Light hors d'oeuvres served</i>	ORSINI

WEDNESDAY, MAY 13, 2015

TIME	EVENT	LOCATION
7:30 AM – 12:00 PM	Registration	REGISTRATION FOYER
8:30 AM – 9:00 AM	Antonin Holý Award Lecture	ESTENSI – MEDICI
9:15 AM – 11:30 AM	Antiviral Chemistry	ESTENSI – MEDICI
11:30 AM – 12:00 PM	Shotgun Presentation I	ESTENSI – MEDICI
12:00 PM – 1:30 PM	Career Networking and Discussion Luncheon	ORSINI
12:00 PM	Networking Afternoon	

THURSDAY, MAY 14, 2015

TIME	EVENT	LOCATION
7:30 AM – 5:30 PM	Registration	REGISTRATION FOYER
8:30 AM – 9:15 AM	William Prusoff Young Investigator Award	ESTENSI – MEDICI
9:15 AM – 12:00 PM	Emerging Viruses Symposium <i>Sponsored by Antiviral Research</i>	ESTENSI – MEDICI
12:00 PM – 1:15 PM	Lunch	On Your Own
1:15 PM – 1:30 PM	ISAR Business Meeting	ESTENSI – MEDICI
1:30 PM – 3:30 PM	Oral Sessions – Targets and Mechanism of Action	ESTENSI – MEDICI
3:30 PM – 4:00 PM	Shotgun Presentations II	ESTENSI – MEDICI
4:00 PM – 6:00 PM	Poster Session 2 <i>Light hors d'oeuvres served</i>	FERNADES
7:30 PM – 8:00 PM	Closing Reception	FARNESE AND ORSINI
8:00 PM – 10:00 PM	Closing Banquet	FARNESE AND ORSINI

FRIDAY, MAY 15, 2015

TIME	EVENT	LOCATION
7:30 AM – 12:00 PM	Registration	REGISTRATION FOYER
8:30 AM – 10:00 AM	Keynote Address	ESTENSI – MEDICI
11:00 AM – 12:00 PM	Oral Sessions – Animal Models	ESTENSI – MEDICI



ICAR Career Networking and Discussion Luncheon

Wednesday, May 13, 2015

12:00 PM – 1:30 PM | ROOM: ORSINI

Please join us for an informal career discussion and networking at the 28th ICAR meeting. This year we will again host an excellent group of moderators who are recognized experts in various areas of antiviral research and have pursued successful career in academia, government, or industry. As a part of the informal group discussion, the moderators will be ready to share their experience, answer questions, and provide feedback about career development. In addition, you will have opportunity to network with your colleagues and make new contacts.

The event is open to all ICAR attendees. Please sign up during the ICAR conference at the registration desk before 10:30 AM on Tuesday, May 12.

Moderators

ACADEMIA SECTOR MODERATORS

- Jose Este (Irsicaixa, Spain)
- Piet Herdewijn (University of Leuven, Belgium)

GOVERNMENT SECTOR MODERATORS

- Sina Bavari contacted (USAMRIID, USA)
- Ramya Natarajan, PhD (NIH, USA)

BIOTECH SECTOR MODERATORS

- Randall Lanier, PhD (Chimerix, USA)
- Sven Enterlein (IBT, USA)

LARGE PHARMA SECTOR MODERATORS

- Weidong Zhong (Novartis, USA)
- Robert Jordan (Gilead, USA)
- Richard Mackman (Gilead, USA)

CONTRACT RESEARCH ORGANIZATIONS (CRO) SECTOR MODERATORS

- J. Robert Bostwick (SRI, USA)
- Eric Stavale (IBT, USA)



Monday, May 11
11:30 – 2:00 pm

ROOM: ORSINI

PARCO DEI PRINCIPI HOTEL
Rome Italy

3rd Annual WOMEN IN SCIENCE ROUNDTABLE

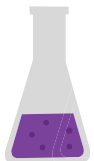
This session is open to all ICAR attendees, both women and men, and will feature prominent women scientists who will talk about the challenges they have faced and the lessons they have learned while navigating the twists and turns of their personal career progression. We invite you to come and hear their perspectives as well as network with other scientists in the industry, government and academic sectors.

The following speakers are confirmed:



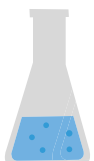
Professor Gabriella Campadelli-Fiume

Microbiology and Virology, University of Bologna,
Department of Experimental, Diagnostic and Specialty Medicine, Bologna, Italy



Professor Kathie Seley-Radtke

Chemistry and Biochemistry, University of Maryland, Baltimore County,
Baltimore, MD, USA



Professor Jennifer Moffat

Microbiology and Immunology, State University of New York (SUNY)
Upstate Medical University, Syracuse, NY, USA



WOMEN IN SCIENCE WINNERS

2015 International Society for Antiviral Research (ISAR) Women in Science (WIS) Career Development Award

2015 International Society of Antiviral Research (ISAR) – Women in Science (WIS) Committee is excited to announce the winners of its 2015 Career Development Award. This award supports the professional development of women with potential for significant contribution in the field of Antiviral Research.

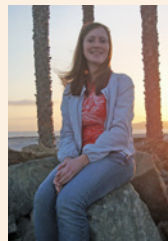
2015 ISAR-WIS AWARDEES



Carol-Ann Eberle
SANOFI

Carol-Ann is currently working as a post-doc at Sanofi in Lyon, a position which is supported by the Marie Curie INBIONET consortium. She received her BA (honors) degree in Molecular Medicine from Trinity

College in Dublin in 2010, followed by a Ph.D. which she conducted at the Centre for Molecular Medicine in Vienna, focusing on virus-host protein interactions.



Lydia Meador
Arizona State University

Lydia graduated with her bachelor's degree in Botany and Microbiology from Oklahoma State University in 2011. She is currently pursuing her Ph.D. in Biological Design at Arizona State University where her work

focuses on developing vaccines for diseases such as HIV and Ebola using viral vectors and plant-produced virus-like particles.



Anastasia Hyrina
University of British Columbia

Anastasia Hyrina is a Ph.D. candidate at the University of British Columbia studying the interplay between dengue and hepatitis C viruses and host lipid metabolic pathways. She is a scholar of the Canadian National

CIHR Research Training Program in Hepatitis C and is highly involved in teaching and community outreach activities.



Kristina Prachanronarong
University of Massachusetts Medical School

Kristina graduated from Brown University and is currently pursuing an MD/Ph.D. degree at the University of Massachusetts Medical School through the Medical

Scientist Training Program. She is studying antiviral drug resistance and antibody neutralization escape mechanisms in influenza under the mentorship of Dr. Celia Schiffer.



Cecilia Martin-Gandul
University Hospital Virgen del Rocío

Cecilia Martin-Gandul obtained her undergraduate degree in Pharmaceutical Sciences from the University of Seville, Spain in 2002, and then joined the University Hospital Virgen del Rocío in Seville as a predoctoral researcher, where she basically worked on translational research on cytomegalovirus infection in transplant patients. In 2014, she completed her Ph.D. in Biomedical Sciences also at the University of Seville.



PROGRAM SCHEDULE

MONDAY, MAY 11, 2015

Women in Science Roundtable

Chair(s): **Amy Patick, Ph.D.**

ORSINI

11:30 AM – 2:00 PM

Drug Development 101

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

ESTENSI – MEDICI

2:00 PM – 3:30 PM

- 2:00 PM** **1. Drug At the Right Place and Right Time: Complexities of Prep PK/PD.**
Betsy Herold, M.D.
Albert Einstein College of Medicine, Bronx, NY
- 2:20 PM** **2. Perceptibility: Understanding User-Centered Experiences in Drug Development.**
Kathleen Morrow, Ph.D.
Brown University, Providence RI
- 2:40 PM** **3. Utilizing Nonhuman Primate SIV Models to Understand The Biology of Primary HIV Infection.**
Jacob Estes, Ph.D.
National Cancer Institute, Frederick MD
- 3:00 PM** **4. An In Vitro Sterilization Assay to Predict the Required Dose of a Microbicide Product.**
Karen W. Buckheit, M.S.
ImQuest Biosciences, Frederick MD
- 3:20 PM** **5. Discussion.**
Robert Buckheit, Jr., Ph.D.
ImQuest Biosciences, Frederick MD

Coffee Break

FERNANDES

3:30 PM – 4:00 PM

Welcome

Robert Buckheit, Ph.D.

ESTENSI – MEDICI

4:00 PM

Keynote Addresses

Chair(s): **Jose Este, Ph.D.**

ESTENSI – MEDICI

4:15 PM – 6:15 PM

4:15 PM

6. From the Elucidation of the HCV Life Cycle to the Development of Highly Effective Antivirals.

Raffaele De Francesco, Ph.D.

Istituto Nazionale Genetica Molecolare, Milano, Italy

KEYNOTE

5:15 PM

7. Doctors Without Borders Perspective on Ebola.

Armand Sprecher, M.D. M.P.H.

Doctors Without Borders, Brussels, Belgium

KEYNOTE

Opening Reception

FARNESE AND ORSINI

6:15 PM – 8:15 PM

TUESDAY, MAY 12, 2015

RNA Viruses Symposium

Chair(s): **Mike Bray, M.D. and Heiner Wedemeyer, M.D.**

ESTENSI – MEDICI

8:30 AM – 12:00 PM

8:30 AM

8. Cruising with Norovirus: Progress and Challenges in Antiviral Drug Discovery.

Joana Rocha-Pereira, Ph.D.

Rega Institute for Medical Research, Leuven, Belgium

9:00 AM

9. Developments in Antivirals to Prevent Rabies.

Anthony Fooks, Ph.D.

Animal Health and Veterinary Laboratories Agency, Carlisle, UK

9:30 AM

10. Therapeutics for Dengue: Current State and Future Directions.

James Whitehorn, Ph.D.

London School of Hygiene & Tropical Medicine, London, UK

Coffee Break

FERNANDES

10:00 AM – 10:30 AM

10:30 AM 11. Hepatitis E: Need for New Therapies.**Heiner Wedemeyer, M.D.***Hannover Medical School, Hannover, Germany***11:00 AM 12. HCV Entry Inhibitors.****Thomas Baumert, M.D.***University of Strasbourg, Strasbourg, France***11:30 AM 13. Development of Antivirals Against Respiratory Syncytial Virus.****John De Vincenzo, M.D.***University of Tennessee, Memphis, United States***Lunch Break**

ON YOUR OWN

12:00 PM – 1:30 PM

Gertrude Elion Award LectureChair(s): **Robert Buckheit, Ph.D.**

ESTENSI – MEDICI

1:30 PM – 2:30 PM

Sofosbuvir: A Search For A Cure**Phillip Furman, Ph.D.****Drug Screening Technologies**Chair(s): **Tim Block, Ph.D and Dimitrios Topalis, Ph.D.**

ESTENSI – MEDICI

2:30 PM – 3:15 PM

2:30 PM 14. Development of Viral Entry Inhibitors Against MERS-CoV and Ebola Virus.**Shibo Jiang^{1,2}***¹Shanghai Medical College, Fudan University, Shanghai, China, ²Lindsley F. Kimball Research Institute, New York Blood Center, New York, United States***2:41 PM 15. A Cell-Based High Throughput Assay for the Discovery of Compounds with Antiviral or Innate Immune Response-Modulating Activities.****Fang Guo¹**, Xuesen Zhao², Yanming Du², Andrea Cuconati², Michael Goetz², Timothy M. Block^{1,2}, Ju-Tao Guo¹, Jinhong Chang¹*¹Drexel University College of Medicine, Doylestown, Pennsylvania, United States, ²Baruch S. Blumberg Institute, Hepatitis B Foundation, Doylestown, Pennsylvania, United States***2:52 PM 16. Discovery of a Broad Spectrum Antiviral Specifically Targeting a Novel Virus-Host Interaction.****Donghoon Chung¹**, Chad Schroeder², Scott Adcock¹, Jarrod Pennington¹, Jennifer Golden²*¹University of Louisville, Louisville, Kentucky, United States, ²University of Kansas, Lawrence, Kansas, United States*

3:03 PM 17. Definition of Key Residues in Viral DS RNA Recognition by Ebola Virus Vp35: Insights for Drug Development.

Enzo Tramontano¹, Valeria Cannas¹, Simona Distinto¹, Luca Zinzula¹, Valeria Fadda², Gian Luca Daino¹, Garry Taylor², Elias Maccioni¹

¹Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy, ²Biomedical Sciences Research Complex, School of Biology, University of St Andrews, St Andrews, United Kingdom

Coffee Break

FERNANDES

3:15 PM – 3:45 PM

In Vitro Evaluation and Antiviral Resistance

Chair(s): **Graciela Andrei, Ph.D. and Justin Julander, Ph.D.**

ESTENSI – MEDICI

3:45 PM – 4:30 PM

3:45 PM 18. Pharmacodynamic Investigation of a Human Rhinovirus Inhibitor in a Hollow Fiber Infection Model.

Qin Yu, Renu Singh, Abhishek Sathe, and Kenneth D. Johnson.

AstraZeneca R&D, Infection iMed Unit, Waltham, MA, United States

3:55 PM 19. Mechanism of Action and Resistance to an EV71 Pocket-Binding Compound.

James T Kelly¹, Luigi De Colibus², Elizabeth E Fry², David I Stuart², David J Rowlands¹, Nicola J Stonehouse¹

¹University of Leeds, Leeds, West Yorkshire, United Kingdom, ²University of Oxford, Oxford, Oxfordshire, United Kingdom

4:05 PM 20. Direct Antiviral Effects of Various Pathogen Receptor Agonists in HBV-Replicating Hepatocytes.

Julie Lucifora^{1,2}, Sarah Maadadi^{1,2}, Océnae Floriot^{1,2}, Marc Bonnin^{1,2}, Stéphane Daffis³, Simon Fletcher³, Fabien Zoulim^{1,2,4}, David Durantel^{1,2}

¹INSERM U1052, Lyon, Rhône-Alpes, France, ²University of Lyon, Lyon, Rhône-Alpes, France, ³Gilead Sciences, Foster city, CA, United States, ⁴Hospices Civils de Lyon (HCL), Lyon, Rhône-Alpes, France

4:15 PM 21. Resistance to Nucleotide Analogues in HPV-Positive and HPV-Negative Cells Emerges Through a Multifactorial Process.

Dimitrios Topalis, Tatiane Cristina Nogueira, Lieve Naesens, Graciela Andrei, Robert Snoeck

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

Poster Session I

FERNANDES

4:30 PM – 6:30 PM

22. Study of γ -Substituted Nucleoside Triphosphate Analogues as Potential Nucleosidic Phosphoantigens.

Javier Alguacil¹, Eric Champagne², Christian Perigaud¹, Suzanne Peyrottes¹

¹UMR5247 CNRS-University Montpellier, Montpellier, France, ²Centre de Physiopathologie de Toulouse Purpan, Toulouse, France

23. Competitive-Fitness Studies of Drug-Resistant Herpes Simplex 2 (HSV-2) DNA Polymerase Mutants.

G. Andrei¹, S. Gillemot¹, F. Morfin², G. Opdenakker¹, R. Snoeck¹

¹Rega Institute, KU Leuven, Leuven, Belgium, ²Hospices Civils de Lyon, Lyon, France

24. Pyrazole Derivatives as Novel Antiflavivirus Compounds.

Elena M. Atzori¹, Nicoletta Desideri¹, Rossella Fioravanti¹, Antonio Carta², Irene Briguglio², Cristina Ibba³, Ilenia Delogu³, Roberta Loddo³

¹Università di Roma, Roma, RM, Italy, ²Università di Sassari, Sassari, SS, Italy, ³Università di Cagliari, Cagliari, CA, Italy

25. Synthesis of Natural Product Mimetic as Possible Anti-HSV Agent.

Chandralata Bal¹, Harapriya Chakravarty¹, Debprasad Chattopadhyay², Ashoke Sharon¹

Department of Chemistry, BIT Mesra, Ranchi, Jharkhand, India, ²ICMR Virus Unit, ID & BG Hospital, Kolkata, West Bengal, India

26. Synthesis and Evaluation of Octadecyloxyethyl Benzyl 9-[(2-Phosphonmethoxy) Ethyl]Guanine (ODE-Bn-PMEG), a Potent Inhibitor of HPV DNA Amplification.

James R. Beadle¹, Nadejda Valiaeva¹, Hsu-kun Wang², Guang Yang², Louise T. Chow², Karl Y. Hostetler¹

¹University of California, San Diego, La Jolla, CA, United States, ²University of Alabama at Birmingham, Birmingham, AL, United States

27. Correlation Between Side Arm Dipole Moments and Human CD4 Down-Modulating Abilities in Unsymmetrical Cyclotriazadisulfonamide (CADA) Compounds with Anti-HIV Activities.

Thomas W. Bell¹, Reena Chawla¹, Victor van Puyenbroeck², Dominique Schols², Kurt Vermeire²

¹Department of Chemistry, University of Nevada, Reno, NV, United States, ²Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

28. Effects of Nonnucleoside HIV-1 Reverse Transcriptase Inhibitors on the Initiation of (+)-Strand DNA Synthesis.

Gilberto Betancor¹, Mar Álvarez¹, Barbara Marcelli¹, Cristina Andrés^{1,2}, Miguel A Martínez², Luis Menéndez-Arias¹

¹Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, 28049, Spain, ²Fundació irsiCaixa, Badalona, Barcelona, Spain

29. Activity of Antiviral Compounds in Cells Model of Virus Co-Infection.

Liubov Biliavska, Olga Povnitsa, Svitlana Zagorodnya, Nadiya Nesterova

Institute of Microbiology and Virology NAS of Ukraine, Kyiv, Ukrenia

31. New Approaches in Detecting Different Causative Agents and Prediction of Disease Progression.

V.P. Bocharnikov¹, B.R. Bogomolnyy², V.P. Barzinskiy², T.L. Grydina³, S.V. Sveshnikov¹

¹Consulting group, Kiev, Ukrenia, ²Corporation Information Medicine, Kiev, Ukrenia, ³I. I. Mechnikov Ukrainian Anti-plague Institute, Odesa, Ukrenia

32. Optimizing HTS Assays to Screen for Antiviral Agents.

J. Robert Bostwick, E. Lucile White, Nichole Tower, N. Miranda Nebane, Shalisa Sanders, Lynn Rasmussen

Southern Research, Birmingham, Alabama, United States

33. Design, Synthesis and Biological Evaluation of Human DDX3 Inhibitors with Multiple Antiviral Activities.

Maurizio Botta

Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, Siena, SI, Italy

34. Inhibitory Effect of Cidofovir on Parvovirus B19 Replication.

Gloria Bua, Francesca Bonvicini, Elisabetta Manaresi, Giorgio Gallinella

University of Bologna, Dept. of Pharmacy and Biotechnology, Bologna, BO, Italy

35. Structure-Based Identification and Chemical Optimization of Anti-HCV Pyrazolobenzothiazine Derivatives Targeting NS5B.

Rolando Cannalire¹, Giuseppe Manfroni¹, Maria Letizia Barreca¹, Pieter Leysen², Johan Neyts², Neerja Kaushik-Basu³, Jan Paeshuyse², Violetta Cecchetti¹

¹Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia, Perugia, PG, Italy, ²Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium, ³Department of Biochemistry and Molecular Biology, Rutgers—The State University of New Jersey, Newark, New Jersey, United States

36. N-[4-(1H(2H) Benzotriazol-1(2)-Yl)Phenyl]Alkylcarboxamides: a New Class of Antiviral Agents.

Antonio Carta¹, Irene Briguglio¹, Paola Corona¹, Nicoletta Desideri², Elena M. Atzori², Ilenia Delogu³, Gabriella Collu³, Roberta Loddo³

¹Università di Sassari, Sassari, SS, Italy, ²Sapienza Università di Roma, Roma, RM, Italy, ³Università di Cagliari, Cagliari, CA, Italy

37. Mouse Lung Slices: an Ex Vivo Model for Evaluation of Antiviral and Anti-Inflammatory Agents Against Influenza Viruses.

Xulin Chen¹, Rui Liu¹, Liwei An^{1,2}, Ge Liu¹, Wei Tang¹

¹Wuhan Institute of Virology, CAS, Wuhan, Hubei province, China, ²The University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region, China

38. Molecular Epidemiology of Emerging Crimean Congo Haemorrhagic Fever Virus in Iran.

Sadegh Chinikar¹, Nariman Shahhosseini², Tahmineh Jalali¹, Sahar Khakifirouz¹

¹Arboviruses and Viral Haemorrhagic Fevers Laboratory (National Ref. Lab), Department of Virology, Pasteur Institute of Iran, Tehran, Tehran, Iran, ²WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Hamburg, Germany

39. Characterization of the Dynamics of HBV Resistances and Genetic Diversity from Longitudinally Antiviral Therapy Treated Patients with Next Generation Sequencing.

Yoon-Seok Chung, Hui-Seong Kim, Hyojin Yang, Hyun Park, Byeong-Sun Choi, Chun Kang

Division of AIDS, Center for Immunology and Pathology, Korea National Institute of Health, Chungju-si, Chuncheongbuk-do, South Korea

40. Phenotypic Characterization of HIV-1 Reverse Transcriptase Associated Activities of a Multiresistant Subtype Ag Strain.

Angela Corona¹, Anna Schneider², Jochen Bodem³, Bernd Buchholz⁴, Elias Maccioni¹, Roberto Di Santo⁵, Enzo Tramontano¹, Birgitta M Wöhr²

¹University of Cagliari, Cagliari, Italy, ²University of Bayreuth, Bayreuth, Germany, ³University of Würzburg, Würzburg, Germany, ⁴University of Mannheim, Mannheim, Germany, ⁵Sapienza University of Rome, Rome, Italy

41. Structural Optimization of the Cycloheptathiophene-3-Carboxamide Scaffold to Target HIV-1 Ribonuclease H.

Jenny Desantis¹, Angela Corona², Serena Massari¹, Giuseppe Manfroni¹, Francesca Esposito², Violetta Cecchetti¹, Enzo Tramontano², Oriana Tabarrini¹

¹Department of Pharmaceutical Sciences, Perugia, Italy, ²Department of Life and Environmental Sciences, Cagliari, Italy

42. Ester Prodrugs for the Delivery of Anti-Filoviral N-Alkyldeoxyribovirin Ihvr-19029.

Yanming Du¹, Huagang Lu¹, Fang Guo², Julia Ma², Bill Kinney¹, Timothy Block^{1,2}, Jinhong Chang²

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43. Nonclinical Pharmacokinetics and Preferential Respiratory Tract Distribution of GS-5806, Fusion Inhibitor of Respiratory Syncytial Virus.

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45. Pharmacokinetics and Preclinical Characterization of GS-5806, Oral Respiratory Syncytial Virus Inhibitor, in Adult and Neonate Preclinical Species.

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46. The Antiviral Drug Discovery and Development Center (AD3C): an Academic Drug Discovery Consortium for (Re-) Emerging Viral Infections.

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47. New Thiosemicarbazones Derived from 1-Indanones with Antiviral Activity Against Bovine Viral Diarrhea Virus.

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48. Computer-Aided Discovery of Small-Molecules Against Norovirus.

Salvatore Ferla¹, Marcella Bassetto¹, Sebastiano Ferla^{1,2}, Salvatore Guccione², Johan Neyts³,

Joana Rocha-Pereira³, Pieter Leyssen³, Andrea Brancale¹

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KU Leuven, Belgium

49. Development of a High-Throughput Screening Assay for Alphavirus RNA Capping Inhibitors.

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50. Antiviral Action of the Benzodiazoles' Derivatives.

T. Grydina¹, I. Koltsova¹, A. Fedchuk², V. Lozitski², S. Basok³, L. Muryk², L. Shitikova², L. Socheslo²

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51. Recombinant Neuraminidase Proteins for Identification of Potential Markers of Drug Resistance in Avian Influenza A (H7N9) Viruses.

Larisa Gubareva, Sleeman Katrina, Zhu Guo, Hua Yang, Erin Hodges, Vasilii Mishin, Julie Villanueva, James Stevens

Centers for Diseases Control (CDC), Atlanta, GA, United States

52. Characterization of the Dynamics of Human Cytomegalovirus Resistance to Antiviral Drugs by Ultra-Deep Sequencing.

Hélène Guermouche¹, Mélanie Mercier-Darty¹, Sonia Burrel², Thomas Kofman³, Olivier Rogier¹, Jean-Michel Pawlotsky¹, Christophe Rodriguez¹, David Boutolleau²

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53. Co- Circulation of Hepatitis A, B and C with Hepatitis E Virus Genotype 1A During Major Outbreak of 2014 in Nepal.

Birendra Prasad Gupta

Central Department of Biotechnology, Kathmandu, Kathmandu, Nepal

54. In Vitro Anti-Dengue Activity of Flavonoids.

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55. Impact of Amino Acid (AA) Substitutions in Influenza Virus Neuraminidase (NA) on Resistance Development Against an Orally Bioavailable Amidine Derivative of Oseltamivir.

Anja Hoffmann¹, Dennis Schade², Johannes Kirchmair³, Andreas Sauerbrei¹, Michaela Schmidtke¹

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56. 's-Monophosphates of 's-Deoxyuridine Derivatives Bearing Extended C-5 Alkynyl Fragments: Synthesis and Biological Properties.

Karpenko Inna, Alexandrova Liudmila

Engelhardt Institute of Molecular Biology RAS, Moscow, Russia

57. Leveraging Your Strengths with Strategic Alliance Management.

Raj Kalkeri, Ashis Saha, Christopher Locher

BioLexUS, Lexington, MA, United States

58. Bio-Activity Guided Fractionation and Characterization of Anti HSV-1 Molecule from Nilgirianthus Ciliatus Nees (Acanthaceae).

Anjana Karunakaran Nair, Prasanth Francis, Manal Mohammed, Moolajogi Nanjan Chandrasekar, Ashish Wadhvani
JSS College of Pharmacy, Ootacamund, Tamilnadu, India

59. Efficacy of Isolated Molecules from Nilgirianthus Ciliatus Nees (Acanthaceae) Against Herpes Simplex Virus Type-1.

Anjana Karunakaran Nair, Prasanth Francis, Manal Mohammed, Moolajogi Nanjan Chandrasekar, Ashish Wadhvani
JSS College of Pharmacy, Ootacamund, Tamilnadu, India

60. Regioselective Synthesis of Pyrazolo[3,4-D]Pyrimidine Based Carbocyclic Nucleosides as Possible Antiviral Agents.

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¹Department of Chemistry, BIT Mesra, Ranchi, Jharkhand, India, ²Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan

61. Development of in Silico Drug Resistance Evaluation Systems for Anti-HIV Drugs Based on the Target Protein Structures.

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62. Identification of a Broad-Spectrum Inhibitor of RNA Viruses.

Samira Khair¹, Marianne Lucas-Hourani¹, Olivier Helynck², H  l  ne Munier-Lehmann², Audrey Page^{3,4}, Sylvain Baize^{3,4}, Fr  d  ric Tangy¹, Pierre-Olivier Vidalain¹
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63. Acyclovir 5'-Hydrogenphosphonate: Antiherpetic Properties and Interaction with Other Nucleoside Drugs.

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64. Isolation and Characterization of Neutralizing Single Domain Antibodies Against Junin Virus.

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65. Potent Broad Spectrum Anti-DNA Viral Activity for HPMPA and HPMPA Tyrosinamide Prodrugs.

Elke Lipka¹, Charles McKenna², Boris Kashemirov², Melissa Williams², Carol Hartline³, Emma Harden³, Geraldine Jefferson³, Kathy Keith³, and Mark N. Prichard³

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66. Structure-Based Design of Novel Thiadiazoloacrylamides as Flavivirus Protease Inhibitors.

Hailong Liu^{1,2,3}, Linlin Zhang^{1,3}, Wint W. Phoo⁴, Dahai Luo⁴, Subhash Vasudevan⁵, Rolf Hilgenfeld^{1,2,3}, Xu Shen², Hong Liu²

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67. New 4(1H)-Quinolinone Derivatives to Defeat Chikungunya Virus.

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68. Testing Anti-HIV Activity of Antiretroviral Agents In Vitro Using Infection of CEM-GFP Cells with an HIV-1 NI4-3 Recombinant Strain and Flow Cytometry.

Beatrice Macchi¹, Caterina Frezza¹, Sandro Grelli², Maurizio Federico³, Francesca Marino-Merlo⁴, Antonio Mastino^{4,5}

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⁵The Institute of Translational Pharmacology, CNR, Rome, Italy

69. Oral Respiratory Syncytial Virus (RSV) Fusion Inhibitor GS-5806: Potency and Oral Pharmacokinetics Optimization.

Richard L Mackman¹, Michael Sangi¹, David Sperandio¹, Jay P Parrish¹, Eugene Eisenberg¹, Michel Perron¹, John DeVincenzo², Tomas Cihlar¹

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70. Identification of a Hybrid Small Molecule That Potently Disrupts the Polymerase PA-PB1 Subunits Interaction and Shows Broad Anti-Flu Activity.

Serena Massari¹, Jenny Desantis¹, Giulio Nannetti², Laura Goracci³, Beatrice Mercorelli², Gabriele Cruciani³, Arianna Loregian², Oriana Tabarrini¹

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71. Anti-Influenza Activity of a New Coordination Compounds.

M.V. Matyushkina¹, T.L. Grydina¹, I.I. Seifullina², V.V. Godovan¹, E.E. Martsinko², E.A. Chebanenko², L.M. Mudryk³, A.S. Fedchuk³

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72. A New Rapid and Accurate Method for Screening Plants Extracts for Antiviral Activity.

Sigal Matza Porges, Kobi Eisen, Hadeel Ibrahim, Adva Haberman, Tal Haimov, Liana Govani, Bertold Fridlender, Gili Joseph

Hadassah-Academic college, Jerusalem, Israel

73. Selective Inhibitors of PI4K II β as Broad Spectrum Antiviral Agents.

Ivana Mejdrová¹, Dominika Chalupská¹, Martin Kögler¹, Michal Šála¹, Pavla Pláková¹, Gabriel Birkus², Evzen Boura¹, Radim Nencka¹

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74. Poly-Functional Immune Response in HIV Infection: a Correlation Between Immunological and Clinical Status.

Serena Meraviglia¹, Giuliana Guadagnino², Paola Di Carlo², Diego Pampinella³, Claudia Imburgia², Francesco Dieli¹

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75. Antiviral Activity of the Medicinal Serum Containing Metabolites of Baicalein as Anti-Dengue Virus Agent.

Ehsan Moghaddam¹, Teoh Boon-Teong¹, SAMSing-Sin¹, Rafidah Lani¹, Zamri Chik², Andrew Yueh³, Sazaly Abubakar¹, Keivan Zandi¹

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76. Synthesis and In-Vitro Antimicrobial Activity of Novel 2-Nitro Bezoic Acid Derivatives.

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77. Purposeful Search and Structural Drug Design of New Anti-Influenza Agents Using Hit QSAR.

Eugene Muratov², Anatoly Artemenko¹, Ekaterina Varlamova¹, Liudmila Ognichenko¹, Stepan Basok¹, Alekseeva Elena¹, Alla Fedtchouk³, Victor Kuz'min¹

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78. In Vitro Selection and Characterization of Influenza A Virus Variants Resistant to a Novel Polymerase Inhibitor of PA/PB1 Interaction.

Giulio Nannetti¹, Giulia Muratore¹, Beatrice Mercorelli¹, Laura Goracci², Gabriele Cruciani², Paul Digard³, Giorgio Palù¹, Arianna Loregian¹

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79. Antiviral Activity of Natural Phytochemicals Against Noroviruses.

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80. Activity of a Triple Antiviral Combination Administered by Consecutive Alternative Treatment Scheme Against Coxsackievirus B1 Infection in Mice.

Ivanka Nikolova, Adelina Stoyanova, Angel S. Galabov

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

81. Preliminary Evaluation of Anti-Poliovirus Effect of Two Lactic Acid Bacteria in an In Vitro Assay System.

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82. Selective Inhibition of HIV-1 Replication in Latently Infected Cells by the CDK9 Inhibitor Fit-039.

Mika Okamoto¹, Akemi Hidaka¹, Masaaki Toyama¹, Makoto Yamamoto², Masatoshi Hagiwara², Masanori Baba¹

¹Kagoshima University, Kagoshima, Kagoshima, Japan, ²Kyoto University, Kyoto, Kyoto, Japan

83. Formation of Efavirenz Cocrystals from Stoichiometric Solutions of Incongruently Saturating Systems by Spray Drying Technology.

Jaywant N. Pawar, Purnima D. Amin

Institute of Chemical Technology, Mumbai, Maharashtra, India

84. Design and Synthesis of New 1-Phenyl-5-(1H-Pyrrol-1-Yl)-1H-Pyrazole-3-Carboxamides as Anti-HCV Agents Targeting Cyclooxygenase-2.

Sveva Pelliccia¹, Giuseppe La Regina¹, Dinesh Manvar², Neerja Kaushik-Basu², Johan Neyts³, Romano Silvestri¹

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85. Novel Potent Antiviral Derivatives Against Poliovirus.

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86. Design, Synthesis and Anti-Corona Virus Activity of a Series of Acyclic Fleximer Analogues.

Hannah L. Peters¹, Dirk Jochmans², Adriaan H. de Wilde³, Clara C. Posthuma³, Eric J. Snijder³, Johan Neyts², Katherine L. Seley-Radtke¹

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87. Synthesis and In Vitro Assay of Deoxyhypusine Synthase Inhibitors as Potential Anti-HIV Agents.

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88. Modeling the Evolution of Acyclovir Resistance with an In Vitro Compartment Model and Viral Dynamic Modeling.

Mark Prichard¹, Scott James¹, Caroll Hartline¹, Emma Harden¹, Kun Wang², Edward Acosta¹

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89. Tryptophan Derivatives That Bind HIV Glycoproteins Gp120 and Gp41.

Ana San-Félix¹, Eva Rivero-Buceta¹, Elisa G. Doyagüez^{1,2}, Leen Mathys³, SAMNoppen³, Sandra Liekens³, María-José Camarasa¹, Jan Balzarini³

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90. Limonoids from Melia Azeradach Fruits as Inhibitors of Flaviviruses.

Giuseppina Sanna¹, Silvia Madeddu¹, Gabriele Giliberti¹, Filippo Cottiglia², Nikoleta Ntalli², Pierluigi Caboni²

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91. Some Basic Principle for the Drug Resistance Preventive Antivirals Design.

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92. 5-(Heteroaryl)-Isoxazole: a New Scaffold Optimization as Possible Anti-HIV Agent.

Ashoke Sharon¹, Debashis Mitra², Afsana Parveen¹, Chandralata Bal¹

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93. Thiophosphoramidate Prodrugs: the Discovery of ALS-2200.

David B Smith, Jerome Deval, Natalia Dyatkina, Hua Tan, Qingling Zhang, Guangyi Wang, Julian Symons, Leonid Beigelman

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94. The Design, Synthesis and Antiviral Activity of New Acyclic Nucleoside Phosphonates Bearing Unsaturated Fragments in the Chain.

Pavel N. Solyev, Maxim V. Jasko, Marina K. Kukhanova

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95. Effect of the Anti-Enteroviral Combination of Pleconaril, MDL-860 and Oxoglaucine Applied in Consecutive Alternating Administration (CAA) Course in Cocksackievirus B1 Neuroinfection in Mice.

Adelina Stoyanova¹, Ivanka Nikolova¹, Gerhard Puerstinger², Georgi Dobrikov³, Vladimir Dimitrov³, Stefan Philipov³, Angel S. Galabov¹

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96. Comparative Study of the Antiviral Activity of Broad-Spectrum and Enterovirus-Specific Inhibitors Against Clinical Isolates of Enterovirus-D68.

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97. Hydroxylated Tropolones as Lead Compounds for Novel Anti-Hepatitis B Virus Drugs.

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98. Preclinical Studies with Novel Herpesvirus Inhibitors from Two Chemical Classes.

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99. Targeting Protein-Protein Interactions as a Successful Therapeutic Strategy Against Viral Infectious Diseases.

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100. Rhodanine Derivatives as Potent Anti-HIV Microbicides.

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101. New Antiviral Molecule from Phyllocaulis Boraceiensis Mucus to Treat Measles.

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Mariana Tonelotto¹, Ronaldo Zucatelli Mendonça¹

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102. Selective Inhibition of HBV Replication by Novel Neplanocin A Derivatives.

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103. Influenza Virus and RSV Virospot & Trade; Assays for High-Throughput Virology Testing.

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104. Identification and Synthesis of 1,4-Disubstituted Piperidines as New Entry Inhibitors of H1N1 Influenza Virus.

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105. Dengue Virus (DENV) Serotype and Endothelial Cell Type/Origin Determine Endothelial Dysfunction After DENV Infection.

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106. Stereoselective Synthesis of Iso-Carbocyclic Nucleoside Analogues.

Simon Weising, Chris Meier

University of Hamburg, Hamburg, Germany

107. Efficient Synthesis of Ribavirin-Phosphoramidites for Biochemical Applications.

Matthias Winkler¹, Ivo Sarac¹, Cristina Ferrer-Orta², Núria Verdaguer², Chris Meier¹

¹University of Hamburg, Hamburg, Germany, ²Molecular Biology Institute of Barcelona, Barcelona, Spain

108. Development and Application of Pseudotype Virus for Antiviral Drug Screening Against Highly Pathogenic Avian Influenza Virus (HPAI) Under Biosafety Level 2 (BSL2) Facility.

Hwajung Yi, Junhyung Cho, Kisoong Kim

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109. In Vitro Antiviral Activity of Scutellaria Baicalensis Against Dengue Virus.

Keivan Zandi¹, Tong-Hye Lim², Nor-Aziyah Rahim¹, Meng-Hooi Shu¹, Boon-Teong Teoh¹, Sing-Sin Sam¹, Kim-Kee Tan¹, Sazaly Abubakar¹

¹Tropical Infectious Disease Research and Education Center (TIDREC), Department of Medical Microbiology, Faculty of Medicine, University Malaya, Kuala Lumpur, W. Persekutuan, Kuala Lumpur, Malaysia, ²Herbitech Sendirian Berhad, G-3-7, Plaza Damas Jalan Sri Hartamas, Sri Hartamas, Kuala Lumpur, Malaysia., Kuala Lumpur, W. Persekutuan, Kuala Lumpur, Malaysia

110. Novel Flutimide Analogues Targeting the Influenza Virus PA Endonuclease.

Grigoris Zoidis¹, Erofilia Giannakopoulou¹, Annelies Stevaert², Vassilios Myrianthopoulos¹, Emmanuel Mikros¹, Lieve Naesens²

¹Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Athens, Attiki, Greece,

²Rega Institute, Katholieke Universiteit Leuven, Leuven, Belgium

111. Novel Phenyl(2-(Phenylamino)Pyrimidin-4-Yl)Methanones as Potent Non-Nucleoside Reverse Transcriptase Inhibitors.

Petr Šimon¹, Ondřej Baszczynski¹, David Šaman¹, Gina Bahador², George Stepan², Eric Hu², Eric Lansdon², Petr Jansa², and Zlatko Janeba¹

¹IOCB, Prague, Czech Republic, ²Gilead Sciences, Inc, Foster City, CA, California, United States

WEDNESDAY, MAY 13, 2015

Antonin Holý Lecture Award

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

ESTENSI – MEDICI

8:30 AM – 9:15 AM

Discovery of Novel Therapeutics for Treating Various Types of Viral Diseases, Cancers, and Inflammatory Disorders

Dennis Liotta, Ph.D.

Emory University, USA

Antiviral Chemistry

Chair(s): **Chris Meier, Ph.D. and Kathie Seley-Radtke, Ph.D.**

ESTENSI – MEDICI

9:15 AM – 11:30 AM

9:15 AM 112. Design, Synthesis and Biological Evaluation of Human DDX3 Inhibitors with Multiple Antiviral Activities.

Maurizio Botta, Ph.D.

Università degli Studi di Siena, Siena, Italy

9:45 AM 113. Design and Pharmacological Characterization of Novel Channel Blockers Targeting the Drug-Resistant M2 Proton Channels from the Influenza A Viruses.

Jun Wang, Chunlong Ma, Fang Li

University of Arizona, Tucson, AZ, United States

Coffee Break

FERNANDES

10:00 AM – 10:30 AM

10:30 AM 114. Triphosphate Prodrugs (Trippro'S) of Biologically Active Nucleosides.

Chris Meier¹, Tristan Gollnest¹, Tobias Nack¹, Domenique Schols², Jan Balzarini²

¹*Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany,*

²*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium*

10:45 AM 115. Indolylarylsulfones Carrying a Heterocyclic Tail as Very Potent and Broad Spectrum HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors.

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

¹*Sapienza University of Rome, Rome, Italy,* ²*Cardiff University, Cardiff, CF, United Kingdom,* ³*Universitat*

Autònoma de Barcelona, Badalona, B, Spain

11:00 AM 116. Molecular Modelling Studies on the Ternary Complex of Dengue Virus Polymerase.

Cecilia M. Cima, Salvatore Ferla, Joachim J. Bugert, Andrea Brancale

Cardiff University, Cardiff, Wales, United Kingdom

11:15 AM 117. Triazolopyrimidines are Able to Efficiently Inhibit Chikungunya Virus Replication: Synthesis and SAR.

Maria-Jesus Perez-Perez¹, Alba Gigante¹, Leen Delang², Gilles Querat³, Dirk Jochmans², Pieter Leyssen², Maria-Jose Camarasa¹, Eva-Maria Priego¹, Johan Neyts²

¹Instituto de Quimica Medica (IQM-CSIC), Madrid, Spain, ²Laboratory for Virology and Experimental Chemotherapy, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium, ³UMR190, Emergence des Pathologies Virales, Aix-Marseille University, Marseille, France

Shotgun Presentations I

Chair(s): **Mark Prichard, Ph.D.**

ESTENSI – MEDICI

11:30 AM – 12:00 PM

11:30 AM 118. Inactivation of Hepatitis B Virus in Chronically Infected Cells Using a CRISPR/Cas9 Nickase RNA-Guided Endonuclease.

Jan Chemnitz¹, Madina Karimova¹, Niklas Beschorner¹, Werner Dammermann², AdAMGrundhoff^{1,3}, Frank Buchholz⁴, Julian Schulze zur Wiesch^{2,3}, Joachim Hauber^{1,3}

¹Heinrich Pette Institute – Leibniz Institute for Experimental Virology, 20251 Hamburg, Germany, ²Department of Medicine I, University Medical Center Eppendorf, 20246 Hamburg, Germany, ³German Center for Infection Research (DZIF) partner site Hamburg, Hamburg, Germany, ⁴Department of Medical Systems Biology, University Hospital and Medical Faculty Carl Gustav Carus, TU Dresden, 01307 Dresden, Germany

11:35 AM 119. Design, Synthesis and Anti-Corona Virus Activity of a Series of Acyclic Fleximer Analogues.

Hannah Peters¹, Dirk Jochmans², Adriaan de Wilde³, Clara Posthuma³, Eric Sniijder³, Johan Neyts², Katherine Seley-Radtke¹

¹University of Maryland Baltimore County, Baltimore, M.D., United States, ²Rega Institute, University of Leuven, Leuven, Belgium, ³Leiden University Medical Center, Leiden, Netherlands

11:40 AM 120. Repositioning a Hepatitis C Virus Antiviral Small Molecule to Treat Dengue Infection.

Ilane Hernandez-Morales, Peggy Gelyukens, Marleen Clynhens, Rudy Strijbos, Erwin Coesemans, Benoit De Boeck, Kenneth Simmen, Frederik Pauwels, Jan Martin Berke, Koen Vandyck, Pedro Lory, Marnix Van Loock
Janssen Infectious Diseases and Vaccines, Beerse, Belgium

11:45 AM 121. Alpha-Carboxy Nucleoside Phosphonates as Universal Nucleoside Triphosphate Mimics.

Matthias Götte¹, Jan Balzarini², Kalyan Das³, Lieve Naesens², Anita R. Maguire⁴, Eddy Arnold³

¹University of Alberta, Edmonton, Alberta, Canada, ²Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ³Centre for Advanced Biotechnology and Medicine, Picataway, New Jersey, United States, ⁴University College Cork, Cork, Cork County, Ireland

11:50 AM 122. Efficacy of a DNA-Based Live Attenuated Vaccine Against Yellow Fever Virus in a Hamster Model of Disease.

Justin G. Julander¹, Niraj Mishra², Jung Ae-Choi¹, Dieudonne B. Kum², Johan Neyts², Kai Dallmeier²

¹Institute for Antiviral Research, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, United States, ²Laboratory of Virology, Rega Institute for Medical Research, KU Leuven-University of Leuven, Leuven, Belgium

Career Networking and Discussion Luncheon

FARNESE

12:00 PM – 1:30 PM

THURSDAY, MAY 14, 2015

William Prusoff Young Investigator Award

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

ESTENSI – MEDICI

8:30 AM – 9:15 AM

Remodel, Repurpose, Rearrange: Essential Forms and Functions Created by the Hidden Viral Proteome

Erica Ollman Saphire, Ph.D.

The Scripps Research Institute, USA

Emerging Viruses Symposium

Chair(s): **Johan Neyts, Ph.D and Anna Papa, M.D., Ph.D.**

ESTENSI – MEDICI

9:15 AM – 12:00 PM

9:15 AM 123. Antiviral Research Awards.

Mike Bray, M.D.

NIAID/NIH, Bethesda, MD

9:30 AM 124. Experimental Therapies for Ebola Virus Infections: What Has Been Learned.

Mike Bray, M.D.

NIAID/NIH, Bethesda, MD

Coffee Break

FERNANDES

10:00 AM – 10:30 AM

10:30 AM 125. Globalization of Chikungunya: 10 Years to Invade the World.

Remi Charrel, M.D., Ph.D.

Aix-Marseille Université Marseille, Marseille France

11:00 AM 126. Emerging Viruses in the Balkans and the Mediterranean Region.

Sponsored by Antiviral Research

Anna Papa, M.D., Ph.D.

Aristotle University of Thessaloniki, Thessaloniki, Greece

11:30 AM 127. Middle East Respiratory Syndrome.

Bart Haagmans, M.D., Ph.D.

Erasmus MC, Zuid Holland, Netherlands

Lunch Break

On your own

ISAR Business Meeting

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

ESTENSI – MEDICI

1:15 PM – 1:30 PM

Antiviral Targets and Mechanism of Action

Chair(s): **Leen Delang, Ph.D and Mark Prichard, Ph.D.**

ESTENSI – MEDICI

1:30 PM – 3:30 PM

- 1:30 PM 128. Evaluation of an Adenovirus Vectored Filovirus Vaccine for Efficacy Against Marburg Virus Angola Aerosol Challenge of Cynomolgus Macaques.**
Aysegul Nalca¹, Elizabeth Zumbrun¹, Holly Bloomfield¹, Benoit Callendret³, Bridget Lewis²
¹Center for Aerobiological Sciences, USAMRIID, Frederick, Maryland, United States, ²Pathology Division, USAMRIID, Frederick, Maryland, United States, ³Crucell Holland B.V. (Janssen Pharmaceuticals), Leiden, Netherlands
- 1:42 PM 129. A Novel Nuclear Transport Inhibitor That Provides In Vivo Protection Against Lethal Dengue Virus Infection.**
David A. Jans¹, Johanna E Fraser¹, Satoru Watanabe², Chunxiao Wang¹, Wing Ki Kitti E Chan², Kylie M Wagstaff¹, Patrick Sexton³, Subhash Vasudevan²
¹DBMB, Monash University, Monash, Victoria, Australia, ²ProgrAMin Emerging Infectious Diseases, Duke-NUS, Graduate Medical School, Singapore, ³Monash Institute of Pharmaceutical Sciences, Monash Uni., Parkville, Victoria, Australia
- 1:54 PM 130. An Induced-Fit Binding Model for Nucleozin-Mediated Influenza A Nucleoprotein (NP) Aggregation.**
Namnam Cheung^{1,2}, Jun Dai^{1,2}, Fang Yang^{1,2}, Richard Y Kao^{1,2}
¹Department of Microbiology, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, ²Center of Infection and Immunology, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong
- 2:06 PM 131. New Structural Data and Molecular Dynamics Simulations on Hepatitis C Virus NS5B Illuminate Activation and Nucleotide Analog Inhibition of Viral RNA Polymerases.**
Yves Boulard^{1,2}, Stéphane Bressanelli¹
¹Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France, ²iBiTec-S, Gif-sur-Yvette, France
- 2:18 PM 132. Biochemical Characterization of the Interaction Between ALS-8112 Triphosphate and the RNA-Dependent RNA Polymerase of Respiratory Syncytial Virus.**
Jerome Deval¹, Jin Hong¹, Guangyi Wang¹, David Smith¹, Rachel Fearn², Sushmita Chanda¹, Julian Symons¹, Leo Beigelman¹
¹Alios BioPharma, South San Francisco, CA, United States, ²Boston University, Boston, MA, United States
- 2:23 PM 133. Combating Norovirus-Dependent Gastroenteritis Through RdRp Inhibitors.**
Eloise Mastrangelo¹, Delia Tarantino², Romina Croci², Jacques Rohayem³, Claudio Nastruzzi⁴, Jih Ru Who⁵, Martino Bolognesi^{1,2}, Mario Milani¹
¹Institute of Biophysics, CNR, Milano, Italy, ²Dep. of Biosciences, University of Milano, Milano, Italy, ³Riboxx GmbH, Pharmapark Radebeul, Radebeul, Germany, ⁴Dep. of Life Sciences, University of Ferrara, Ferrara, Italy, ⁵Dep. of Chemistry, National University, Zhongli, Taiwan

Coffee Break

FERNANDES

2:30 PM – 3:00 PM

- 3:00 PM 134. Evaluation of the Histone Deacetylase Inhibitor ST7612A1 as an HIV-1 Latency Reactivation Agent.**
Roger Badia¹, Judith Grau¹, Eva Riveira-Muñoz¹, Bonaventura Clotet¹, Ester Ballana¹, Giuseppe Gianini², José Esté¹
¹AIDS Research Institute – IrsiCaixa, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Catalonia, Spain, ²R&D Sigma-Tau Industrie Farmaceutiche Riunite SpA, Via Pontina km30, 400, I-00040, Pomezia, Roma, Italy
- 3:05 PM 135. Quinoxaline-6-Carboxamides Inhibit HBV Infection In Vitro.**
Vadim Bichko, Alexei Rjakhovskiy, Eugenia Remeeva, Boris Rogovoy
 Viriom Inc., San Diego, CA, United States
- 3:10 PM 136. A Novel Class of Chikungunya Virus Inhibitors Targets the Enzymatic Activity of the Viral Capping Enzyme NSP1.**
Leen Delang¹, Changqing Li², Ali Tas³, Martijn van Hemert³, Maria Jesús Pérez-Pérez⁴, Bruno Coutard², Johan Neyts¹, Pieter Leyssen¹, Gilles Quérat⁵
¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²UMR7257 CNRS, Aix Marseille University, Marseille, France, ³Molecular Virology Laboratory, Leiden University Medical Center, Leiden, Netherlands, ⁴Instituto de Química Médica (IQM-CSIC), Madrid, Spain, ⁵UMR190, Emergence des Pathologies Virales, Aix-Marseille University, France
- 3:15 PM 137. A Novel Class of Host-Directed Antivirals with Broad Spectrum Activity Against Respiratory and Systemic RNA Viruses.**
Shari M Kaiser¹, Jhoanna Noonan¹, Nathan A Hedin¹, Kerry Fowler¹, Michael Gale Jr², Shawn P Iadanato¹, Kristin M Bedard¹
¹KINETA, Inc., Seattle, WA, United States, ²University of Washington, Dept. Microbiology and Immunology, Seattle, WA, United States
- 3:20 PM 138. T-705 and Ribavirin Induce Lethal Mutagenesis of Influenza Virus.**
Evelien Vanderlinden¹, BrAMVrancken¹, Jeroen Van Houdt², Graciela Andrei¹, Philippe Lemey¹, Lieve Naesens¹
¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²Centre for Human Genetics, University Hospital Leuven, KU Leuven, Leuven, Belgium
- 3:25 PM 139. Discovery of Dengue Virus NS4B Inhibitors.**
Quing-Yin Wang¹, Hongping Dong¹, Bin Zou¹, Paul Smith¹, Pei-Young Shi¹,
 Novartis Institute for Tropical Diseases, Singapore, Singapore

Shotgun Presentations II

Chair(s): **Andrea Brancale, Ph.D. and Kathie Seley-Radke, Ph.D.**

ESTENSI – MEDICI

3:30 PM – 4:00 PM

To Be Announced

Poster Session II

FERNANDES

4:00 PM – 6:00 PM

141. Studies on Anti-HIV Activity, Cytotoxicity of Wrightia Tinctoria R. Br. Leaf.

P Selvam¹, Akhil C. Banerjee²

¹ Nova College of Pharmaceutical Education and Research, Jupudi, Krishna DT, A.P, India, ²Virology Laboratory National Inst. Immunology, New Delhi-110067, India

- 142. Evasion of Innate Immunity Mediated by Orf Virus Protein Ov20.0.**
 Yeu-Yang Tseng¹, Fong-Yuan Lin¹, Sun-Fang Cheng¹, Chia-Chi Chou², Min-Liang Wong², Wei-Li Hsu¹
¹Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung, Taiwan,
²Department of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan
- 143. The Antiviral Effect of Favipiravir (T-705) on Coxsackievirus B3 Replication Is Modulated by the Nature of the Amino Acid Residue At the Highly Conserved Position 159 of the RNA-Dependent RNA Polymerase.**
 Rana Abdelnabi¹, Pieter Leyssen¹, Bruno Canard², Johan Neyts¹, Leen Delang¹
¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²AFMB, Aix-Marseille Université, Marseille, France
- 145. Development and Characterization of Surface Modified Chitosan Nanoparticles for Selective Targeting of Lamivudine to Hepatocyte.**
 Gomed Agarwal¹, Saurabh Bhargava², Vishal Bhargava¹
¹KRV Hospitals Pvt. Ltd., Kanpur, U.P., India, ²Manav Bharti University, Kanpur, U.P., India
- 146. Lipid Based Nanoparticulate System for Effective Vaccine Delivery.**
 Gomed Agarwal¹, Saurabh Bhargava², Vishal Bhargava¹
¹KRV Hospitals Pvt. Ltd., Kanpur, U.P., India, ²Manav Bharti University, Kanpur, U.P., India
- 147. Antibody Coated Liposomes for Transmucosal Vaccination.**
 Manee Agarwal¹, S Bhargava²
¹ICFAI University, Kanpur, U.P., India, ²Manav Bharti University, Kanpur, U.P., India
- 148. SMADs, IRFs and Antiviral Innate Immunity: Defining the Intersection of BMP and IFN Signalling at the Genomic Level.**
 Kinda Al-Hourani, Lucy Eddowes, Hal Drakesmith MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, Oxfordshire, United Kingdom
- 149. Triple Mode of Anti-Viral Action of Verapamil Against Influenza Virus Replication.**
 Intakhab Alam¹, Pumaree Kanrai¹, Julia Dzieciolowski¹, Ahmed Mostafa¹, Mueller Christin¹, John Ziebuhr¹, Ursula Dietrich², Stephan Pleschka¹
¹Institute of Medical Virology, University of Giessen, Giessen, Hessen, Germany, ²Georg-Speyer-Haus, Frankfurt, Hessen, Germany
- 150. Suramin Inhibits Chikungunya Virus Replication Through Multiple Mechanisms.**
 Irina Albuлесcu, Marcella van Hoolwerff, Ali Tas, Florine Scholte, Eric Snijder, Martijn van Hemert
 Department of Medical Microbiology, Leiden University Medical Center, Leiden, ZH, Netherlands
- 151. Uptake and Metabolism of the HPV Antiviral, Octadecyloxyethyl Benzyl 9-[2-(Phosphonomethoxy)Ethyl]Guanine, in HFF and HEK-293 Cells.**
 Kathy A. Aldern¹, James R. Beadle¹, Nadejda Valiaeva¹, Karl Y. Hostetler^{1,2}
¹University of California, San Diego, La Jolla, CA, United States, ²Hera Therapeutics Inc, Del Mar, CA, United States
- 152. Uptake and Metabolism of the HPV Antiviral, Octadecyloxyethyl Benzyl [8-14C] 9-[2-(Phosphonomethoxy)Ethyl]Guanine, in Hff and Hek-293 Cells.**
 Kathy A. Aldern, James R. Beadle, Nadejda Valiaeva, Karl Y. Hostetler
 Univ California San Diego, La Jolla, CA, United States, United States

- 153. Inhibition of Herpes Simplex Virus Type 1 (HSV-1) by the Cdk6 Inhibitor PD-0332991 (Palbociclib) Through the Control of SAMHD1.**
Guillem Angulo¹, Roger Badia¹, Eva Riveira-Muñoz¹, Ester Ballana¹, Bonaventura Clotet¹, Eui Tae Kim², Matthew D Weitzman², José A Esté¹
¹AIDS Research Institute-IrsiCaixa, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Catalonia, Spain, ²Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman Medical School and Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States
- 154. Relationship Between Mutations in E2 Proteins and Their Response to Interferon Alpha 2B Plus Ribavirin Therapy in HCV Genotype 3A Pakistani Patients.**
Binish G Arshad¹, Abida Raza², Javaid Irfan², Samina N Shakeel¹
¹Quaid-i-Azam University, Islamabad, Pakistan, ²Nuclear Medicine, Oncology and Radiotherapy Institute, Islamabad, Pakistan
- 155. Antiviral Activity of a G-Quadruplex Ligand Against Herpes Simplex Virus-1.**
Sara Artusi¹, Ilaria Frasson¹, Rosalba Perrone¹, Arianna Calistri¹, Louis Flamand², Giorgio Palu¹, Sara N. Richter¹
¹Department of Molecular Medicine, University of Padua, Padua, Italy, ²Department of Microbiology, Infectious Diseases and Immunology, University of Laval, Quebec, Quebec, Canada
- 156. Chemokine (CCL2, CXCL10) and Interleukin (IL28B, IL10) Gene Variability and Human Predisposition to Tick-Borne Encephalitis.**
Andrey V. Barkhash¹, Mikhail I. Voevoda^{1,2}, Aida G. Romaschenko¹
¹Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia, ²Institute of Internal Medicine SB RAMS, Novosibirsk, Russia
- 157. Biochemical Characterization of the PA ,PA-X and the Natural PA-X Deletion Mutant of Human Influenza A Virus.**
Laura Bavagnoli, Stefano Cucuzza, Giovanni Maga
 Inst. of Molecular Genetics IGM-CNR, Pavia, Italy
- 158. Non-Invasive Topical Immunization Using Cholera Toxin as Adjuvant for the Treatment of Hepatitis B.**
M Bhargava¹, S Bhargava², V Bhargava³
¹ICFAI University, Kanpur, U.P., India, ²Manav Bharti University, Kanpur, U.P., India, ³KRV Hospitals Pvt. Ltd., Kanpur, U.P., India
- 159. Combining Hepatitis B Surface Antigen with Tetanus for a Single Oral Vaccine.**
S Bhargava¹, V Bhargava²
¹Manav Bharti University, Kanpur, U.P., India, ²KRV Hospitals Pvt. Ltd., Kanpur, U.P., India
- 160. Linear and Hyperbranched Polyglycerol Based Multivalent Glycoarchitectures as Influenza Virus Inhibitors.**
Sumati Bhatia¹, Daniel Lauster², Kai Ludwig³, Stefano A. Uberti⁴, Andreas Herrmann², Rainer Haag¹
¹Institut für Chemie und Biochemie, Organische Chemie, Freie Universität Berlin, Takustr. 3, 14195 Berlin, Germany, ²Institut für Physik, Physik von Makromolekülen, Humboldt-Universität zu Berlin, Invalidenstr. 42, 10115 Berlin, Germany, ³Institut für Chemie und Biochemie, Zentrum für Elektronenmikroskopie Berlin, Newtonstr. 15, Berlin, Germany, ⁴Institut für Physik, Humboldt-Universität zu Berlin, Fabeckstr. 36a, 14195 Berlin, Germany, ⁵Germany, ⁶Germany

161. TAOK3 Phosphorylates the Methylenecyclopropane Nucleoside MBX 2168 to its Monophosphate.

Terry L. Bowlin¹, Gloria Komazin-Meredith¹, Steven C. Cardinale¹, Katelyn Comeau¹, Caroll B. Hartline², John D. Williams¹, Timothy J. Opperman¹, Mark N. Prichard²

¹Microbiotix, Inc., Worcester, MA, United States, ²University of Alabama at Birmingham, Birmingham, AL, United States

162. Antiviral Activity of AGMA1 Polymer Against Human Papillomaviruses and Preclinical Study as a Topical Microbicide.

Valeria Cagno¹, Manuela Donalizio¹, Roberta Cavalli¹, Marco Rusnati², David Lembo¹

¹University of Turin, Turin, TO, Italy, ²University of Brescia, Brescia, BS, Italy

163. Efficacy of Replication-Defective Lymphocytic Choriomeningitis Virus Vectors (rLCMV) Expressing Guinea Pig Cytomegalovirus Antigens Against Congenital Cytomegalovirus Infection in Guinea

Pigs.R. D. Cardin¹, FJ Bravo¹, DA Pullum¹, K Orlinger², A Aspoeck², G Fuhrmann², M Schwendinger², DI Bernstein¹

¹Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States, ²Hookipa Biotech AG, Vienna, Austria

164. Assessment of Lethality of 1976 Ebola-Zaire and 2014 Ebola-Liberia Strain in a Novel Humanized Mouse Model.

Ayan K. Chakrabarti¹, Brian H. Bird¹, Clifton Drew², Marina Khristova³, Jessica Spengler¹, Sherif Zaki², Stuart T. Nichol¹, Christina F. Spiropoulou¹

¹Viral Special Pathogens Branch, The Centers for Disease Control and Prevention, Atlanta, Georgia, United States,

²Infectious Disease Pathology Branch, The Centers for Disease Control and Prevention, Atlanta, Georgia,

United States, ³Biotechnology Core Facility, The Centers for Disease Control and Prevention, Atlanta, Georgia, United States

165. Ve Approach Formula with Four Prong Attack on Ebola Virus Infection with Antivirals, Innate Immune Booster, Anti-Cytokines and Vaccines Along with the Antivirals Passive Prophylaxis to Stamp Out the Epidemic.

M ChandraMohan¹, P Selvam², D. Sivakumar¹, S.C. Vivekananthan¹, M. Kannan¹

¹Bharat Ratna Kamarajar liver Hospital and Research Centre, Madurai, TN, India, ²Nova College of Pharma. Edu. and Research, Jupudi, Krishna DT, A.P, India

166. An Ethnomedicinal Alkaloid and Its Analogue, Inhibit Immediate Early Transcription of Herpes Simplex Virus.

Debprasad Chattopadhyay¹, Durbadal Ojha¹, Ashoke Sharon²

¹ICMR Virus Unit, ID & BG Hospital, Beliaghata, Kolkata, West Bengal, India, ²Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India

167. Identification of a Broad-Spectrum Inhibitor of Common RNA Respiratory Viruses by High-Throughput Screening.

NAMN. Cheung^{1,2}, Jun Dai^{1,2}, Kin K. Lai^{1,2}, Fang Yang^{1,2}, Richard Y. Kao^{1,2}

¹Department of Microbiology, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong,

²Center of Infection and Immunology, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong

168. Protective Efficacy of VP1-Specific Neutralizing Antibody Associated with a Reduction of Viral Load and Pro-Inflammatory Cytokines in Human Scarb2-Transgenic Mice.

Yen-Hung Chow

National Health Research Institutes, Zhunan, Miaoli, Taiwan

169. Telaprevir-Induced Drug Rash with Eosinophilia and Systemic Symptoms Associated with HHV-6 Active Infection.

Giulia Ciccarese¹, Francesco Drago¹, Francesco Broccolo², Linda Bruzzone³, Aurora Parodi¹

¹Department of Dermatology, University of Genoa, Genoa, Italy, Italy, ²Department of Health Science, University of Milano Bicocca, Monza, Italy, Italy, ³Department of Internal Medicine, University of Genoa, Genoa, Italy, Italy

170. Ribonuclease H/DNA Polymerase HIV-1 RT Dual Inhibitor: Mechanistic Studies on the Allosteric Mode of Action of Isatine-Based Compound Rmnc6.

Angela Corona¹, Rita Meleddu¹, Francesca Esposito¹, Simona Distinto¹, Elias Maccioni¹, Luis Menéndez-Arias², Stuart F J Le Grice³, Enzo Tramontano¹

¹University of Cagliari, Cagliari, Italy, ²CBMSO (CSIC-UAM), Madrid, Spain, ³National Cancer Institute, Frederick MD, United States

171. Optimization of Simeprevir Triple Therapy: a Multivariate Logistic Regression Model Using Baseline Predictors.

G D'Offizi¹, C Cammà², M Schlag³, K Weber³, R DeMasi⁴, K Janssen⁴, J Witek⁴, R Lionetti¹

¹National Institute for Infectious Diseases "L. Spallanzani" I.R.C.C.S, Rome, Italy, ²Section of Gastroenterology, Di.Bi.M.I.S., University of Palermo, Palermo, Italy, ³Janssen-Cilag, Vienna, Austria, ⁴Janssen R&D LLC, Titusville, NJ, United States

172. Identification of FDA-Approved Drugs That Inhibit Middle East Respiratory Syndrome Coronavirus Replication in Cell Culture.

Adriaan H. de Wilde¹, Dirk Jochmans², Clara C. Posthuma¹, Jessika C. Zevenhoven-Dobbe¹, Bernadette van den Hoogen³, Johan Neyts², Eric J. Snijder¹

¹Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands, ²Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium, ³Department of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands

173. Identification of Highly Conserved Residues Involved in the Inhibition of the HIV-1 Integrase by Diketoacid Derivatives.

Francesca Esposito¹, Francesco S. Di Leva², Angela Corona¹, Sylvain Thierry³, Sandro Cosconati⁴, Olivier Delelis³, Roberto Di Santo⁵, Enzo Tramontano¹

¹University of Cagliari, Cagliari, Italy, ²University of Naples "Federico II", Naples, Italy, ³LBPA, ENS, Cachan, France, ⁴DiSTABiF, Second University of Naples, Caserta, Italy, ⁵LaSapienza" University of Rome, Rome, Italy

174. Role of the Mitochondrial RNA Polymerase in the Toxicity of Nucleotide Inhibitors of the Hepatitis C Virus.

Joy Feng, Yili Xu, Ona Barauskas

Gilead Science, Foster City, CA, United States

175. Cellular Promyelocytic Leukemia Protein Is an Important Dengue Virus Restriction Factor.

Federico Giovannoni, Elsa B. Damonte, Cybele C. García

School of Sciences, University of Buenos Aires, Buenos Aires, Buenos Aires, Argentina

176. Small Molecule Inhibitors of the HIV-1 Protein Rev.

Luis González-Bulnes¹, Ignacio Ibáñez², Luis M Bedoya³, Angel Cantero-Camacho¹, Silvia Prado¹, Jose Alcami³, Santos Fustero², Jose Gallego¹

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177. Mp-12 Lacking a Functional NSS Gene Confers Complete Protection Against Lethal Rift Valley Fever Virus Disease in Hamster Models of Vaccine and Post-Exposure Intervention.

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178. A Comprehensive Immunoinformatics and Target Site Study Revealed the Corner-Stone Towards Chikungunya Virus Treatment.

Md. Anayet Hasan¹, M.D.. Arif Khan², Amit Datta¹, M.D.. Habibur Hasan Mazumder¹, Mohammad Uzzal Hossain²

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179. Design of Potential RNAi (miRNA and siRNA) Molecules for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Gene Silencing by Computational Method.

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180. Structural Studies on MERS-CoV Proteins: Basis for Antiviral Drug Discovery.

Rolf Hilgenfeld^{1,2,3}, Daizong Lin^{1,2,3}, Hailong Liu^{1,2,3}, Hong Liu³, Qingjun Ma^{1,2}, Yibei Xiao^{1,2}, Jian Lei^{1,2}

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181. Human Subtilase Ski-1/S1P Is a Master Regulator of the Dengue Virus Lifecycle and a Potential Target for Indirect-Acting Antiviral Agents.

Anastasia Hyrina, François Jean

University of British Columbia, Vancouver, BC, Canada

182. Effect of Nucleic Acid Sequence on DNA Polymerization and NNRTI Inhibitory Mechanisms of HIV-1 Reverse Transcriptase.

O Ihenacho¹, A Huber¹, E Michailidis², K Das³, MA Parniak⁴, K Singh¹, E Arnold^{3,4}, S Sarafianos¹

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³Rutgers University, Piscataway, NJ, United States, ⁴University of Pittsburgh, Pittsburgh, PA, United States

183. Development of Bipolymer Based Novel Nanoparticles in Microsphere System as Vaccine Adjuvant.

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184. Discovery an Antiviral Agent Targeting the Influenza Viral M2 Protein from Target-Free Screening of a Chemical Library.

Yejin Jang, Hye Won Lee, Chonsaeng Kim, Chong-Kyo Lee, Meehyein Kim

Korea Research Institute of Chemical Technology, Daejeon, South Korea

- 185. Isolation and Evaluation of Anti-Viral Activity of Trachyspermum Ammi Plant Extract Against Influenza A Virus Infection.**
Madhu Khanna, Saugat Roy, Latika Sexana
VP Chest Institute, University of Delhi, Delhi, India
- 186. Inhibitory Effect of Peramivir Against Avian Influenza A (H7N9) Virus.**
Masanori Kobayashi¹, Haruaki Nobori¹, Keiichi Taniguchi¹, Akihiko Sato¹, Masatoshi Okamoto², Yoshihiro Sakoda², Hirofumi Sawa², Hiroshi Kida²
¹*Shionogi & Co., Ltd., Sapporo, Japan*, ²*Hokkaido University, Sapporo, Japan*
- 187. Soraphen A: a Broad-Spectrum Antiviral Natural Product with Potent Anti-Hepatitis C Virus Activity.**
George Koutsoudakis¹, Inés Romero-Brey², Carola Berger², Gemma Pérez-Vilaró¹, Paula Monteiro Perin³, Kirsten Harmrolfs^{4,5}, Javier P Martinez⁶, Juana Díez¹
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- 188. Host Protein Disulfide Isomerase Represents a Novel Therapeutic Target for the Treatment of Chikungunya Virus Infection.**
Rose M Langsjoen¹, Shannan L Rossi¹, Maria Kastis², Robert L Seymour¹, Scott C Weaver¹
¹*University of Texas Medical Branch, Galveston, TX, United States*, ²*Ball High School, Galveston, TX, United States*
- 189. Peptidomimetic Inhibition of Host-Targeted Serine Proteases as a Treatment Against Influenza.**
Richard Leduc¹, Éloïc Colombo¹, Émilie Gravel², Antoine Désilets¹, Isabelle Marois², Pierre-Luc Boudreault¹, Éric Marsault¹, Martin Richter²
¹*Department of Pharmacology-Physiology, Université de Sherbrooke, Sherbrooke, Quebec, Canada*, ²*Department of Medicine, Université de Sherbrooke, Sherbrooke, Quebec, Canada*
- 190. Anti-Influenza Virus Activity Mediated by Monoacetylcurcumin.**
Yi-Lin Li¹, Yoshiyuki Mizushima², Jason T.C Tzen³, Sarah M. Richart⁴, Wei-Li Hsu¹
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- 191. Exploring Antiviral and Hsp90-Inhibiting Properties of Marine Alkaloid Analogues Through in Silico Modelling and In Vitro Screening.**
Katja-Emilia Lillsunde¹, Tihomir Tomaši², Danijel Kikelj², Päivi Tammela¹
¹*Centre for Drug Research, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland*, ²*Faculty of Pharmacy, University of Ljubljana, Ljubljana, Slovenia*
- 192. The Role of Pre-Existing Antibodies in Determining the Efficacy of Vaccination in Humans.**
Jenny GH Low¹, Limin Wijaya¹, Eng Eong Ooi²
¹*Singapore General Hospital, Department of Infectious Diseases, Singapore, Singapore*, ²*Emerging Infectious Diseases, Duke NUS Graduate Medical School, Singapore, Singapore*

193. Selective Inhibitor of Nuclear Export (SINE) Compound, Verdinexor Alters New World Alphavirus Capsid Localization, and Reduces Viral Replication in Mammalian Cells.

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²Karyopharm Therapeutics, Newton, MA, United States

194. Inhibition of Cellular ER α -Glucosidases as Broad-Spectrum Strategy Against Acute Virus Infections.

Julia Ma¹, Fang Guo¹, Yanming Du², Timothy M. Block^{1,2}, Ju-Tao Guo¹, Jinhong Chang¹

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

195. Orally Available Pyrazolopyrimidines Effectively Target a Broad Spectrum of Enteroviruses by Blocking Capsid Function.

Vadim A. Makarov¹, Heike Braun², Martina Richter², Olga B. Riabova¹, Johannes Kirchmair³, Nora Seidel², Peter Wutzler², Michaela Schmidtke²

¹A.N. Bach Institute of Biochemistry RAS, Moscow, Russia, ²Jena University Hospital, Department of Virology and Antiviral Therapy, Jena, Germany, ³University of Hamburg, Center for Bioinformatics, Hamburg, Germany

196. Combination Therapy with Neuraminidase Inhibitor Oseltamivir and Polymerase Inhibitor T-705 Extends the Therapeutic Window Against Highly Pathogenic Influenza H5N1 Virus.

Bindumadhav M. Marathe¹, Sook-San Wong¹, Peter Vogel¹, Robert G. Webster¹, Richard J. Webby¹, Isabel Najera², Elena A. Govorkova¹

¹St. Jude Children's Research Hospital, Memphis, TN, United States, ²Roche Innovation Center, Basel, Switzerland

197. Analysis of Serological and Cellular Immune Correlates of Protection Against Yellow Fever Infection Induced by DNA-YFVax, a Novel DNA-Based Live-Attenuated Yellow Fever Vaccine.

Rafael E. Marques^{1,2}, Niraj Mishra¹, Dieudonne B. Kum¹, Johan Neyts¹, Kai H. Dallmeier¹

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198. Expression of HLA-DR on T Lymphocytes in HIV-Positive Patients in Dependence of HIV-1 Tropism.

Natallia V. Matsiyevskaya¹, Dmitry E. Kireev², Marina Y. Dmitryukova², Irina O. Tokunova¹

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199. New Antiviral Properties of 5'-Norcarbocyclic Nucleoside Analogues.

Elena Matyugina¹, Vladimir Valyev-Elliston¹, Mikhail Novikov², Enzo Tramontano³, Sergey Kochetkov¹, Katherine Seley-Radtke⁴, Anastasia Khandzhinskaya¹

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200. Early Inhibition of Human Cytomegalovirus Replication as a Therapeutic Option: Design, Identification, and Characterization of New Anti-HCMV Candidate Drugs with a Novel Mechanism of Action.

Beatrice Mercorelli¹, Anna Luginini², Serena Massari³, David Lembo⁴, Oriana Tabarrini³, Giorgio Gribaudo², Giorgio Palù¹, Arianna Loregian¹

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201. Anti-Tumor Effect of Cidofovir Against Human Papillomavirus Positive and Negative Cells Is Not Exclusively Due to DNA Damage.

Barbara Mertens, Lieve Naesens, Graciela Andrei, Robert Snoeck

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

202. The Homodimerization of Human Papillomavirus Oncoprotein E6 as a Target for the Development of New Anti-HPV Drugs.

Lorenzo Messa¹, Beatrice Mercorelli¹, Gualtiero Alvisi¹, Lawrence Banks², Laura Goracci³, Gabriele Cruciani³, Giorgio Palù¹, Arianna Loregian¹

¹Department of Molecular Medicine, University of Padua, Padua, Italy, Italy, ²International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, Italy, ³Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy, Italy

203. Over Additive Effect of Combination of Oseltamivir and Vitamin E on Oxidative Damages in Liver Caused by Influenza Virus Infection in Mice.

Milka Mileva¹, Dimo Krustev², Albena Alexandrova³, Elina Tzvetanova³, Galina Gegova¹, Angel S. Galabov¹

¹The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, ²Medical University, Medical College “J. Filaretova”, Sofia, Bulgaria, ³Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

204. Spectrum of Activity Testing for Dengue Therapeutics in Ag129 Mice; Proof-Of-Concept Studies with NITD-008.

Gregg N. Milligan, Mellodee M. White, Diana L. Zavala, Richard B. Pyles, Vanessa V. Sarathy, Alan D.T. Barrett, Nigel Bourne

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205. Limited Evolution and (Almost) Clonal Expansion of the Yellow Fever Virus During Lethal Infection in Mice, Implication for Vaccine and Drug Development Against Flaviviruses.

Niraj Mishra, Dieudonne B. Kum, Pieter Leyssen, Johan Neyts, Kai H. Dallmeier

Laboratory of Virology, Rega Institute for Medical Research, KU Leuven – University of Leuven, Leuven, Vlaams Brabant, Belgium

206. Identification of Novel Cellular Targets by Screening a Library of Known Bioactive Molecules and Their Potential Use in Anti-HIV Therapeutic Regimen.

Debashis Mitra, Jay Trivedi

National Centre for Cell Science, Pune, Maharashtra, India

- 207. N-Methanocarbathymidine Is Effective Against HSV-1 and VZV in Mice.**
Jennifer F. Moffat¹, Debra C. Quenelle², Mark N. Prichard², Dongmei Liu¹, Wanda Coombs¹, Deborah J. Collins², Terri L. Rice², Caroll B. Hartline^Y, Robert L. Glazer³, Aquilur Rahman⁴
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- 208. Inhibition of Hepatitis C Virus in Mouse Models by Lipidoid Nanoparticle-Mediated Systemic Delivery of siRNA Against PRK2.**
Jae-Su Moon, Seung-Hoon Lee, Hee Cho, Wooseong Lee, Jong-Won Oh
 Department of Biotechnology, Yonsei University, Seoul, Seoul, South Korea
- 209. Human Beta-Defensins 2 and 3 Bind and Inactivate Intracellular Human Immunodeficiency Virus in Oral Epithelial Cells.**
Michael Morris¹, Rossana Herrera¹, Kristina Rosbe², Aaron Weinberg², Sharof Tugizov¹
¹University of California San Francisco, San Francisco, CA, United States, ²Case Western Reserve University, Cleveland, OH, United States
- 210. Druggable Protein-Protein Interactions as Antiviral Targets for an Innovative Chemotherapeutic Intervention.**
Giulio Nannetti¹, Lorenzo Messa¹, Laura Goracci², Serena Massari³, Gabriele Cruciani², Oriana Tabarrini³, Giorgio Palù¹, Arianna Loregian¹
¹Department of Molecular Medicine, University of Padua, Padua, Italy, ²Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy, ³Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
- 211. Phosphonate Prodrugs of Nucleoside HIV RT Inhibitors.**
Anastasia L Khandazhinskaya, Elena S Matyugina, Marina K Kukhanova, Sergey N Kochetkov.
 Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 119991, Russia
- 212. Regulation of HPV16/18 E6, P53 and ATM in Parental and CDV-Resistant Cells After Cidofovir Treatment.**
Tatiane Cristina Nogueira, Barbara Mertens, Dimitrios Topalis, Robert Snoeck, Graciela Andrei
 Rega Institute for Medical Research – KU Leuven, Leuven, Belgium
- 213. Targeting Intracellular Potassium for the Control of Respiratory Syncytial Virus.**
Michael J Norris^{1,2}, Manpreet Malhi³, Wenming Duan², Jason Maynes^{3,4}, Theo J Moraes^{1,2}
¹Dept. of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Ontario, Canada, ²Dept. of Physiology & Experimental Medicine, SickKids, Toronto, Ontario, Canada, ³Dept. of Molecular Structure & Function, SickKids, Toronto, Ontario, Canada, ⁴Dept. of Anesthesia & Pain Medicine, SickKids, Toronto, Ontario, Canada
- 214. DNA Polymerase Inhibitors Enhance the Anti-Viral Effect of Terminase Inhibitors When Used in Combination Against HCMV.**
Shea/M O'Brien, Brian/G Gentry
 Drake University, Des Moines, Iowa, United States
- 215. Anti-HSV Activity of Pedilanthus Tithymaloides, an Indian Folklore, Through the Inhibition of Toll-Like Receptor 3 Signaling Pathway.**
Durbadal Ojha, Debprasad Chattopadhyay
 ICMR Virus Unit, ID & BG Hospital, Beliaghata, Kolkata, West Bengal, India

216. Efficacy of Alpha – ZAMin the Therapy of HIV Infection Among Individuals Seeking Alternative Therapy in Nigeria.

Olufunmilayo G. Oyero¹, Abdulfattah A. Onifade¹, Andrew P. Jewell³, Bashiru A. Okesina⁴

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217. A G-Quadruplex Forming Aptamer Potently Inhibits HIV-1 Entry into the Host Cell.

Rosalba Perrone¹, Giorgio Palu¹, Christophe Pannecouque², Sara N. Richter¹

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218. Ar-12 – A Novel Broad Spectrum Host Directed Antiviral Drug.

Stefan Proniuk¹, Paul Dent², Emma L. Mohr³, Jacob Yount⁴, Alexander Zukiwski¹

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219. The Lipid Kinase Sphingosine Kinase 2 Is an Essential Host Factor Recruited by Chikungunya Virus During Infection.

St Patrick Reid, Sarah R. Tritsch, Krishna Kota, Chih Y. Chiang, Michael D. Ward, Sina Bavari

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220. Antiviral Activity of MEK/ERK Inhibitor AZD6244 Against Dengue Virus in AG129 Mouse.

Aryádina Ribeiro¹, Leonardo de Oliveira¹, Jonas Albarnaz¹, Alice Torres¹, Scott Parker², Kostantin Doronin², Mark Buller², Cláudio Bonjardim¹

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221. Cyclin D3 Regulates CDK6 Control of the Viral Restriction Factor SAMHD1.

Eva Riveira-Muñoz¹, Ester Ballana¹, Ruiz Alba¹, Javier Torres-Torronteras², Roger Badia¹, Bonaventura Clotet¹, Ramón Martí², José A. Esté¹

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222. Biosynthesis of Methylenecyclopropane Nucleoside Analog Triphosphates in HCMV-Infected Cells.

Hannah E. Sauer¹, John D. Williams², Terry L. Bowlin², John C. Drach³, Brian G. Gentry¹

¹Drake University, Des Moines, IA, United States, ²Microbiotix, Inc., Worcester, MA, United States, ³University of Michigan, Ann Arbor, MI, United States

223. Investigating the Antiviral Effects of Cannabinoids in the 2nd Decade of HAART.

M Selbovitz, R Melamede, R Moore

Cannabis Science, Colorado Springs, Colorado, United States

224. Design, Synthesis, Anti-HIV Activity and Cytotoxicity of Novel Indenoquinoline Derivatives.

P Selvam¹, Erik De Clercq², Christophe Pannecouque²

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225. Investigation of Anti-HIV Activity and HIV Integrase Inhibitory Activity of Polyherbal Extracts.

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226. Design and Synthesis of Novel Isatine-3-Thiosemicarbazone Derivatives as Novel Inhibitors of HIV Integrase/LEDGF Protein-Protein Interaction.

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227. A771726, the Active Metabolite of Leflunomide, as Inhibitor of Junin Virus Replication.

Claudia S. Sepúlveda, Cybele C. García, Elsa B. Damonte

Iquibicen Uba Conicet, Buenos Aires, Argentina

228. Antivirals Design Against HIV, Influenza and Ebola on the Way to Nano-Intervention in the Fusion Type I Machinery.

Alexander Serbin^{1,2}, Boris Bolshikov², Olga Alikhanova¹, Vladimir Tsvetkov²

¹Health RDF, Moscow, Russia, ²Petrochem. Synt. Inst., RAS, Moscow, Russia

229. Comparison of Two Chemiluminescent Immunoassay (CMIA I ECLIA) for Determination of HBsAg, Anti HBs and Anti HBe in Human Serum.

Nafija Serdarevic

Institute for Clinical Chemistry and Biochemistry, Clinics Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

230. Biological Response Modifiers as Enhancers of Oseltamivir Activity Against Influenza Virus Type A/H3N2 In Vivo.

Lora Simeonova¹, Galina Gegova¹, Kirilka Todorova¹, Elitsa Pavlova²

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231. Efficacy of Combinations of Oseltamivir and Naproxen for the Treatment of Influenza A (H1N1) Virus Infections in BALB/c Mice.

Donald F. Smee, Ashley Dagley, Brittney Downs, Justin G. Julander

Institute for Antiviral Research, Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States

232. Verdinexor (Kpt-335), a Selective Inhibitor of Nuclear Export (SINE) Compound, Is a Broad Spectrum Inhibitor of Viral Replication.

Sharon Tamir, Margaret Lee, Yosef Landesman, Robert Carlson, Sharon Shacham

Karyopharm Therapeutics Inc., Newton, MA, United States

- 233. Targeting the Flavivirus Polymerase: a New Class of Non-Nucleoside Inhibitors Mimicking the Stacking Interaction of Two RNA Bases.**
Delia Tarantino¹, Eloise Mastrangelo², Romina Croci¹, Giuseppe Manfroni³, Gilles Querat⁴, Martino Bolognesi¹, Mario Milani²
¹University of Milano, Milano, Italy, ²CNR-IBF, Milano, Italy, ³University of Perugia, Perugia, Italy, ⁴CNRS-AFMB, Marseille, France
- 234. Synthetic Toll-Like Receptor Ligands as Adjuvants in a Recombinant Influenza H1 Hemagglutinin Vaccine and Efficacy Against Homologous Virus Challenge in Mice.**
Bart Tarbet¹, Brett Hurst¹, Tomoko Hayashi², Howard Cottam²
¹Institute for Antiviral Research, Utah State University, Logan, UT, ²Department of Medicine, University of California, San Diego, CA,
- 235. Creating Screening of Inhibitors of Herpes Simplex Virus Type 1 Alkaline Nuclease for Molecular Model.**
Chen Tian, Zhang Lei, Cao Kang, Chen Hongyu, Pan Qu, Hu Jin
 Chengdu Medical College, Chengdu, Sichuan, China
- 236. A Cellular Protein Induces and Stabilizes the HIV-1 LTR G-Quadruplex Conformation, a Key Regulatory Element of Viral Transcription and New Antiviral Target.**
Elena Tosoni¹, Iliaria Frasson¹, Matteo Scalabrin^{1,2}, Rosalba Perrone¹, Elena Butovskaya¹, Matteo Nadai¹, Dan Fabris², Sara N. Richter¹
¹Department of Molecular Medicine, University of Padova, Padova, Non-US/Canada, Italy, ²The RNA Institute, University at Albany-SUNY, Albany, NY, United States
- 237. HCMV UI111A and US27 Synergize to Enhance Signaling of CXCR4.**
Carolyn Tu, Juliet Spencer
 University of San Francisco, San Francisco, California, United States
- 238. Dipeptidyl Peptidase 4 (DPP4) in Mink Supports Entry and Replication of Middle Eastern Respiratory Syndrome Coronavirus.**
Thomas G. Voss, Somanna K Naveen, Gena J. Nichols, Chandrika Kannadka, Michael Patterson, Matthew Kappes, Mei-Chun Chen, Shih-Chao Lin
 SRI International, Harrisonburg, VA, United States
- 239. Activity of Neuraminidase Inhibitors (NAIs) on Influenza Virus Replication in the Presence of Pneumococcal Neuraminidase A and B (NANA and NANb).**
Elisabeth Walther¹, Martina Richter¹, Zhongli Xu¹, Judith M. Rollinger², Johannes Kirchmair³, Andreas Sauerbrei¹, Hans Peter Saluz⁴, Michaela Schmidtke¹
¹Jena University Hospital, Department of Virology and Antiviral Therapy, Jena, Germany, ²University of Vienna, Department of Pharmacognosy, Vienna, Austria, ³University of Hamburg, Center for Bioinformatics, Hamburg, Germany, ⁴Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany
- 240. Viremia Reduction Measurement When Antiviral Treatment Is Started At the Time of Peak Viremia in Mice May Be a More Appropriate Mimic for Conditions in the Clinical Setting for Dengue Fever.**
Satoru Watanabe¹, Kittu W.K. Chan¹, Geoff Dow², Jenny Low³, Subhash G. Vasudevan¹
¹Duke-NUS GMS, Singapore, Singapore, ²60° Pharmaceuticals PLC, Washington DC, WA, United States, ³Singapore General Hospital, Singapore, Singapore

241. Competition Model Between Nucleozin and RNA Binding to Influenza Nucleoprotein.

Fang Yang, Jun Dai, Y.T. Kao
the Univeristy of Hong Kong, HK, HK, Hong Kong

242. Antiviral Activity of New Fluorinated Nucleosides Against Herpesvirus.

Svitlana Zagorodnya¹, Galina Baranova¹, Krystyna Naumenko¹, Yriy Shermolovich², Nadiya Nesterova¹
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243. Flexibility of the S2 Subsite in Alpha- and Beta-Coronavirus Main Proteases Determines Susceptibility to Peptidomimetic Inhibitors.

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244. Piceatannol Prevents HIV Entry into the Cells.

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¹Laboratory of Cellular and Molecular Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg, ²Laboratory of Retrovirology, Luxembourg Institute of Health, Luxembourg, Luxembourg, ³South Eastern Kenya University, Kitui, Kenya

245. Antiviral Effect in Influenza Patients Treated with Metered Dose Inhaler Containing Aprotinin, a Protease Inhibitor.

Oleg P. Zhirnov¹, Elena I. Isaeva¹, Natasha O. Bokova², Nikolai A. Malyshev²
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246. Pharmacological Enhancement of Anti-Viral DNA Polymerase Inhibitors Via Reduction of Endogenous Nucleoside Triphosphates.

Joseph J. Zieminski, Laura E. Vollmer, Brian G. Gentry
Drake University, Des Moines, Iowa, United States

247. Establishing In Vitro Assays and a Robust Mouse Model to Identify Inhibitors of the Zika Virus.

J. Zmurko¹, S. Kaptein¹, R. Elias^{1,2}, E. Verbeken³, J. Neyts¹
¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²Immunopharmacology, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, ³Translational Cell and Tissue Research, KU Leuven, Leuven, Belgium

Closing Reception and Banquet

FARNESE AND ORSINI

7:30 PM – 10:00 PM

FRIDAY, MAY 15, 2015

Clinical Evaluation of Antiviral Therapies

Chair(s): **Michael Manns, M.D.**

ESTENSI – MEDICI

8:30 AM – 10:00 AM

8:30 AM 248. Advances in HCV Therapies.**KEYNOTE****Michael Manns, M.D.***Hannover Medical School, Hannover, Germany***9:15 AM 249. Discovery and Characterization of MK-8876, a Novel Non-Nucleoside Inhibitor of HCV NS5B Which Possesses Broad Genotypic Potency.****Steven W. Ludmerer**, Casey McComas, Anandan Palani, Joseph J. Salata, Ellen Hulschette, Maureen Ho, Arthur Simen, Joan R. Butterson*Merck & Co., Kenilworth, NJ, United States***9:45 AM 250. Quantitative Mutant Analysis of Naturally Occurring V-073-Antiviral Resistant Polioviral Quasispecies Using the Maprec-Assay in Infants Receiving OPV.****Kimberley S.M. Benschop**¹, Joost Verhoeven¹, Gokhan Uslu¹, Edin Jusic¹, Erwin Duizer¹, Marion P.G. Koopmans², Harrie G. van der Avoort¹¹National Institute for Public Health and the Environment, Bilthoven, Utrecht, Netherlands, ²Erasmus Medical Center, Rotterdam, Zuid Holland, Netherlands

Coffee Break

FERNANDES

10:00 AM – 10:30 AM

Animal Models of Infection

Chair(s): **Don Smee, Ph.D. and Heather Greenstone, Ph.D.**

ESTENSI – MEDICI

10:30 AM – 12:00 PM

10:30 AM 251. Integrins Coordinate HSV Entry into the Cell and the Immediate Innate Response.**Gabriella Campadelli Fiume, Ph.D.***University of Bologna, Bologna, Italy***11:00 AM 252. Experimental Respiratory Infection of Hartley Guinea Pigs with Ebola Virus Zaire.****Aysegul Nalca**¹, Holly Bloomfield¹, Elizabeth Zumbun¹, Donald Nichols²¹Center for Aerobiological Sciences, USAMRIID, Frederick, Maryland, United States, ²Pathology Division, USAMRIID, Frederick, Maryland, United States**11:10 AM 253. A Novel Dengue Model in STAT2 Knockout Hamsters.****Justin G. Julander**¹, Sang R. Lee², Ashley Dagley¹, John D. Morrey¹, Zhongde Wang²¹Institute for Antiviral Research, Utah State University, Logan, UT, United States, ²Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, UT, United States

- 11:20 AM 254. Connecting the Dots: Study of West Nile Virus Spread Within the Central Nervous System of Primates as a Foundation for Development of Therapeutic Interventions for Flaviviral Encephalitis.**
Olga A. Maximova¹, John G. Bernbaum², Alexander G. Pletnev¹
¹Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, United States, ²Integrated Research Facility at Fort Detrick, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Frederick, Maryland, United States
- 11:30 AM 255. In Vivo Antiviral Efficacy of Verdinexor in Two Animal Models of Influenza A Virus Infection.**
Olivia Perwitasari¹, Scott Johnson¹, Sharon Shacham², Robert Carlson², Sharon Tamir², Ralph Tripp¹
¹Department of Infectious Diseases, University of Georgia College of Veterinary Medicine, Athens, GA, United States, ²Karyopharm Therapeutics, Newton, MA, United States
- 11:40 AM 256. Antiviral Efficacy of an RSV Fusion Inhibitor in a Bovine Model of RSV Infection.**
Robert Jordan¹, Matt Shao², Richard L. Mackman¹, Michel Perron¹, Tomas Cihlar¹, Mark L. Anderson², Heather McEligot², Laurel J. Gerschwin²
¹Gilead Sciences, Foster City, CA, United States, ²University of California, Davis, Davis, CA, United States
- 11:50 AM 257. Cidofovir, Ganciclovir, and Valganciclovir Inhibit Human Adenovirus Replication and Toxicity in Permissive Immunosuppressed Syrian Hamsters.**
William Wold, Baoling Ying, Ann Tollefson, Jacqueline Spencer, Mark Buller, Karoly Toth
Saint Louis University, St. Louis, MO, United States

Drug Screening Technologies

Chairs: **Tim Block, Ph.D.** and **Dimitrios Topalis, Ph.D.**

2:30 PM – 3:15 PM

ESTENSI – MEDICI

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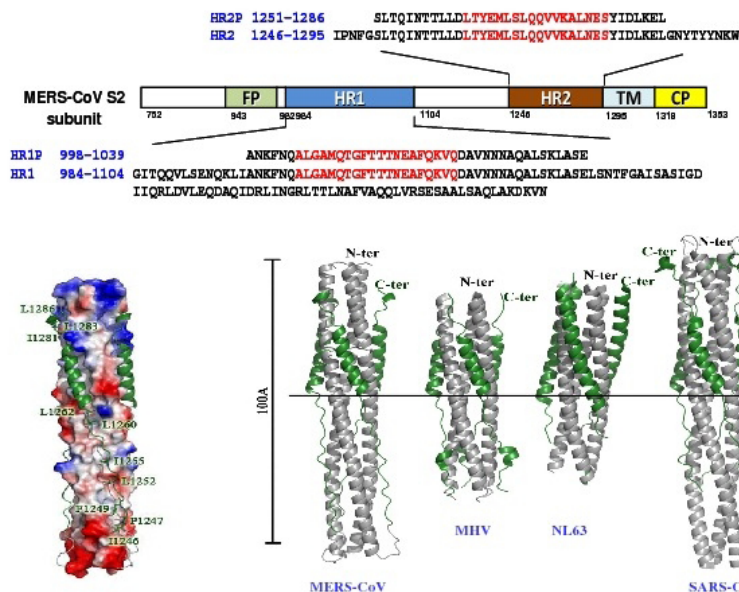
Development of Viral Entry Inhibitors against MERS-CoV and Ebola Virus

Shibo Jiang^{1,2}

¹Shanghai Medical College, Fudan University, Shanghai, China, ²Lindsley F. Kimball Research Institute, New York Blood Center, New York, USA

The epidemics of emerging and re-emerging infectious diseases with high mortality caused by the highly pathogenic viruses, such as Middle East respiratory syndrome coronavirus (MERS-CoV) and Ebola virus (EBOV), have posed great threats to the public health worldwide. Therefore, development of highly effective and safe therapeutics to combat these emerging and re-emerging infectious diseases is urgently needed. Based on our previous experience in developing peptide viral entry inhibitors against HIV (Nature 365:113, 1993) and SARS-CoV (Lancet 363:938-47, 2004), we designed and synthesized a peptide derived from MERS-CoV S protein S2 subunit HR2 domain, designated HR2P, which effectively inhibited MERS-CoV S protein-mediated cell-cell fusion and infection by both pseudotyped and live MERS-CoV with IC50 at low μM level, about 20- to 30-fold more potent than SARS-CoV HR2 peptide against SARS-CoV replication (Nat. Commun. 5:3067, 2014). By modifying the sequence of HR2P, we have identified several peptides with improved anti-MERS-CoV activity and pharmaceutical properties. One of these peptides exhibited excellent in vitro and in vivo efficacy against MERS-CoV infection, suggesting a good potential to develop it as a prophylactic and/or therapeutic agent to combat MERS-CoV infection. Using similar approaches, we have identified a number of EBOV entry inhibitors with potent inhibitory activity against pseudoviruses carrying the glycoprotein (GP) of Zaire ebolavirus (ZEBOV) and Sudan ebolavirus (SEBOV) strains. Their mechanisms of action are under investigation.

Core structure of MERS-CoV S protein S2 subunit



15 **A Cell-Based High Throughput Assay for the Discovery of Compounds with Antiviral or Innate Immune Response-Modulating Activities**

Fang Guo¹, Xuesen Zhao², Yanming Du², Andrea Cuconati², Michael Goetz², Timothy M. Block^{1,2}, Ju-Tao Guo¹, Jinhong Chang¹

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

Virus infection is recognized by host pattern recognition receptors (PRRs) to induce interferon and inflammatory cytokine responses that control the virus infection, but on the other side, are occasionally detrimental to the hosts. Therefore, drugs that either inhibit viral replication or modulate virus-induced cytokine response may have a therapeutic potential. The goal of this study was to establish cell-based high throughput screening for compounds with antiviral and/or cytokine response-modulating activities. The system is HEK293 cell stably expressing firefly luciferase under the control of IFN- promoter. Infection of the cells with a panel of viruses activated IFN- promoter, which led to the expression of firefly luciferase quantitatively correlating to the levels of virus replication. Furthermore, the virus-activated IFN- reporter expression in the cells could be inhibited in a dose-dependent manner by known antiviral compounds or inhibitors of PRR signal transduction pathways. Hence, the reporter assay is suitable for simultaneous discovery of antiviral and innate immune response modulating compounds against many different viruses. Using Dengue virus as an example, a small molecule library of 26,900 compounds was screened. In addition to antivirals such as BPU, a benzothiazolyl urea compound that inhibits the entry of multiple viruses of flavivirus, and USDV-001, a benzodiazepine compound that potently and more specifically inhibits dengue and yellow fever virus (but not other 20 viruses tested), we also identified compounds that suppressed virus-induced cytokine response. Furthermore, a pilot screening of a natural product library consisting of 2,000 spectrum collection of crude extracts from plants, bacteria and fungi, followed by bioactivity-guided fractionation and structure analysis, led to the identification of nigericin as an inhibitor of dengue virus. Further preclinical development of selected antiviral compound including SAR, *in vivo* pharmacokinetics, toxicology, and efficacy in animal model is currently under way.

16 **Discovery of a Broad Spectrum Antiviral Specifically Targeting a Novel Virus-Host Interaction**

Donghoon Chung¹, Chad Schroeder², Scott Adcock¹, Jarrod Pennington¹, Jennifer Golden²

¹University of Louisville, Louisville, USA, ²University of Kansas, Lawrence, USA

Despite of the huge impact of emerging viruses on the public health, few antivirals are available to treat the infections of these viruses. This is largely due to the nature of the currently available antivirals; i.e., virus-specific mechanism of action, narrow antiviral spectrum, and emergence of resistance. While broad spectrum antivirals may overcome these limitations, the development of a broad spectrum antiviral has been hampered by undesirable toxic effects or low efficacy.

To address these gaps, we used a high-throughput screen with an alphavirus and discovered a novel class of compound with broad spectrum antiviral activity. An analogue of hit compound inhibited the replication of a range of emerging viruses, including alphaviruses (Venezuelan and Western equine encephalitis virus, and Chikungunya virus), respiratory syncytial virus, vesicular stomatitis virus, lymphocytic choriomeningitis virus, SARS-CoV, West Nile virus, and influenza virus with greater than 1000-fold reduction in progeny virus titer without cytotoxicity to the host cells ($SI_{50} > 50$). A certain class of virus; e.g., Japanese encephalitis virus, however, was not sensitive to the compound, implying the mechanism of action of the compound is targeting a specific cellular pathway commonly exploited by the sensitive viruses. The initial mechanism of action studies showed that the entry of the viruses is the target of the compound. Through an extensive structure-activity relationship study around the structure, we discovered compounds with IC_{50} s of ~10 nM and $SI_{50} > 1000$. The novel compounds will provide an opportunity to develop new broad spectrum antiviral therapeutics as well as to study the virus entry mechanism exploited by the sensitive viruses.

17 **Definition of key residues in viral dsRNA recognition by Ebola virus VP35: insights for drug development**

Enzo Tramontano¹, Valeria Cannas¹, Simona Distinto¹, Luca Zinzula¹, Valeria Fadda², Gian Luca Daino¹, Garry Taylor², Elias Maccioni¹

¹Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy, ²Biomedical Sciences Research Complex, School of Biology, University of St Andrews, St Andrews, United Kingdom

Ebola virus (EBOV) replication and transcription processes lead to the synthesis of viral double-stranded RNA (dsRNA) whose recognition by cytosolic RIG-I-like receptors (RLRs) induces the alpha-beta interferon (IFN- α/β) innate immune response. EBOV VP35 protein, a viral polymerase co-factor, prevents such recognition through its dsRNA binding properties and participates in

suppressing host IFN- α/β production. Hiding viral dsRNA to RLRs recognition, EBOV VP35 is a crucial determinant of EBOV virulence and pathogenesis and, in fact, mutations in VP35 that reduce its IFN-antagonist capabilities are associated to loss of EBOV virulence. Given the specific 5'-ppp dsRNA EBOV VP35 end-capping recognition showed by crystallographic studies, we wanted to define a dsRNA binding site that could be used as target for drug design. Hence, to identify the most critical residues involved in VP35 dsRNA binding, we performed a structure-based alanine scanning of full-length EBOV VP35 amino acid residues reported to have a role in the end-capping interactions, such as F239, Q274, I278, Q279, K319, R322 and K339. Through a biochemical assay assessing EBOV VP35 dsRNA binding ability and a reporter gene assay measuring the IFN-antagonist ability of VP35 in cell cultures, it was found that alanine substitution of selected residues dramatically decreased VP35 dsRNA binding ability and some of these mutants showed to heavily affect EBOV VP35 IFN-antagonist activity. To further evaluate the impact of alanine substitutions on dsRNA binding, we solved the crystallographic structure of the C-terminal dsRNA binding domain of EBOV VP35 I278A mutant. All collected data, complemented by computational studies, allowed defining a site in the end-capping binding surface that can be used to design small molecules to inhibit EBOV VP35 immune suppression functions.

In Vitro Evaluation and Antiviral Resistance

Chairs: **Andrea Brancale, Ph.D.**

3:45 PM – 4:30 PM

ESTENSI – MEDICI

18 Pharmacodynamic Investigation of a Human Rhinovirus Inhibitor in a Hollow Fiber Infection Model

Qin Yu, Renu Singh, Abhishek Sathe, and Kenneth D. Johnson

AstraZeneca R&D, Infection iMed Unit, Waltham, USA

Pharmacodynamic Investigation of a Human Rhinovirus Inhibitor in a Hollow Fiber Infection Model Kenneth D. Johnson, Renu Singh, Abhishek Sathe, and Qin Yu* Infection Innovative Medicines Unit, AstraZeneca R&D Boston, Waltham, MA, United States Human rhinovirus (HRV) HRV is the most common cause of upper respiratory tract infection and it is linked to exacerbations of chronic pulmonary disease and asthma. Antivirals targeting HRV have been demonstrated to reduce cold symptoms and may have the potential to prevent exacerbations associated with HRV infection. Understanding the relationship between inhibitor exposure and the virological response of HRV is important for predicting the optimal dose and dosing intervals necessary to achieve efficacy in patients. To circumvent the lack of a robust in vitro or in vivo HRV model for this purpose, we developed an HRV in vitro hollow-fiber infection model, which enabled simulation of different inhibitor exposure profiles over HRV grown in cultured cells. Using this model, we investigated the pharmacokinetic (PK)-pharmacodynamic (PD) relationship of an HRV 3C protease inhibitor (Compound 1) by analyzing the effect of multiple inhibitor concentrations and dosing intervals on HRV growth. The results demonstrated that HRV inhibition was governed by the exposure time above a minimal concentration (C_{min}), not by the total exposure (AUC) or peak concentration (C_{max}) of this compound. In summary, we have developed the first HRV hollow fiber system reported to date for in vitro PK-PD investigations and provided insight to the PD driver for HRV protease inhibitors.

19 Mechanism of action and resistance to an EV71 pocket-binding compound

James T Kelly¹, Luigi De Colibus², Elizabeth E Fry², David I Stuart², David J Rowlands¹, Nicola J Stonehouse¹

¹University of Leeds, Leeds, United Kingdom, ²University of Oxford, Oxford, United Kingdom

The picornavirus EV71 is the main causative agent of hand, foot and mouth disease and is especially problematic in East Asia. Although usually associated with mild symptoms in children, occasionally it can result in fatal neurological and cardiovascular disorders. At present no therapies are available. The viral capsid comprises three major structural proteins, VP1, VP2 and VP3 and, as in other enteroviruses, VP1 contains a cavity known as the VP1 pocket which harbours a lipid moiety termed “pocket factor”. Expulsion of this molecule following receptor binding allows capsid alterations required for release of the viral genome. Several low molecular weight hydrophobic compounds that tightly bind within the pocket and replace the “pocket factor” have been shown to inhibit viral uncoating and have potential for development as antiviral drugs. Using the skeletons of a class of imidazolidinones with anti-EV71 activity and the crystal structure of the EV71 particle, rational improvements of such inhibitors have been made. Beneficial substitutions within these molecules were predicted and two new compounds synthesized. One ligand is an order of magnitude more potent than the best previously reported inhibitor (IC₅₀ = 25 pM), and is also more soluble. Here, through time of addition studies, we show that the mechanism of action is to inhibit uncoating. We also show that resistance to the compounds can evolve rapidly and is achieved by preventing the compound from binding into the pocket. The mutations responsible for resistance also appear to prevent the natural pocket factor from binding and result in reduced stability of the virus.

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Direct antiviral effects of various pathogen receptor agonists in HBV-replicating hepatocytes

Julie Lucifora^{1,2}, Sarah Maadadi^{1,2}, Océane Floriot^{1,2}, Marc Bonnin^{1,2}, Stéphane Daffis³, Simon Fletcher³, Fabien Zoulim^{1,2,4}, David Durantel^{1,2}

¹INSERM U1052, Lyon, France, ²University of Lyon, Lyon, France, ³Gilead Sciences, Foster city, USA, ⁴Hospices Civils de Lyon (HCL), Lyon, France

To improve HBV therapies, the identification of new classes of drugs is warranted. Small molecules/agonists capable to boost the innate immunity system could play an important role in restoring an efficient specific adaptive immunity against HBV. In this respect, we tested the direct anti-HBV effect of different pattern recognition receptors (PPR) agonists in relevant cell culture models.

Primary human hepatocytes (PHH) and differentiated HepaRG cells (dHepaRG) were infected by HBV, and twice-treated after the establishment of persistent replication with various concentration of different PPR agonists during 7 days. Cytokine production was analyzed by ELISA after 24h of stimulation, and viral replication markers (HBeAg and HBSAg secretion, total intracellular HBV DNA, cccDNA and HBV RNA) were analyzed at the end of dosings by ELISA, qPCR and qRT-PCR respectively. Toxicity was also analyzed. IP-10 and/or IL-6 were secreted by both PHH and dHepaRG 24h after exposure to TLR1/2, TLR3, TLR4, TLR5, TLR2/6, RIG/MDA5, and AIM2 agonists. Moreover, hepatocyte stimulations by these agonists lead to strong and dose-dependent antiviral effects in absence of any toxicity. The best effect was obtained with Pam3ck4 (TLR1/2-L), with notably a very significant effect on cccDNA, associated with a lack of rebound off-treatment. Stimulations with TLR7, TLR8 and TLR9 agonists neither lead to significant IP-10 or IL-6 production, nor had any inhibitory effect on HBV replication, with the notable exception of one TLR7 agonist, for which an antiviral effect was obtained, irrespective of innate immunity, therefore suggesting an interesting off-target effect and uncovering a new cellular pathway that could be hijacked by HBV.

Our data highlight the importance of hepatocyte responses *per se* in the control of HBV replication, and strongly support the recent interest in the development of PRR-based antiviral strategies against HBV.

21

Resistance to nucleotide analogues in HPV-positive and HPV-negative cells emerges through a multifactorial process

Dimitrios Topalis, Tatiane Cristina Nogueira, Lieve Naesens, Graciela Andrei, Robert Snoeck
Rega Institute for Medical Research – KU Leuven, Leuven, Belgium

INTRODUCTION: Human papillomavirus (HPV) is the causative agent of cervical cancer. Its role in the development of head and neck carcinoma has also been reported in many studies. Currently, no drug has been licensed for the treatment of HPV-related diseases but some compounds, such as cidofovir (CDV), exhibit selective antiproliferative activity *in vitro* and *in vivo*.

METHODS: In this study, we analyzed the effects of CDV-resistance acquisition in two HPV⁺ (SiHa_{CDV} and HeLa_{CDV}) and one HPV⁻ (HaCaT_{CDV}) tumor cell lines on several parameters. Measurement of CDV metabolites levels by HPLC using radiolabeled CDV was performed, as well as analysis of the sensitivity profile to several chemotherapeutics. The emergence of multidrug resistance events was investigated by measuring the protein levels of transporters described as key players in drug-resistance or able to interact with CDV (MRP2, P-gp, BCRP and OAT1).

RESULTS: Alterations of CDV metabolism in SiHa_{CDV} and HeLa_{CDV}, but not in HaCaT_{CDV}, emerged *via* impairment of UMP/CMP kinase 1 (UMP/CMPK1) activity, the enzyme responsible for the phosphorylation of (d)CMP, (d)UMP and cytidine analogues used in antiviral or anticancer therapy. Two mutations in UMP/CMPK1, P64T and R134M, were identified in SiHa_{CDV} while down-regulation of UMP/CMPK1 gene was observed in HeLa_{CDV}. Enzymatic studies and protein stability assays showed that these mutations impair the activity of UMP/CMPK1 and destabilized its structure. All three cell lines showed cross-resistance to other ANPs but also to a few structurally unrelated chemotherapeutics whereas hypersensitivity to other anticancer drugs was observed. Modified expression of transporters (MRP2, BCRP and OAT1) in SiHa_{CDV} and/or HeLa_{CDV} was also observed. UMP/CMPK1 and drug transporters were not differentially expressed in HaCaT_{CDV} cells suggesting a different mechanism of CDV-resistance.

CONCLUSIONS: Taken together, these results indicated that emergence of CDV resistance, in HPV⁺ tumor cells, is a multifactorial process that involves key players of the metabolism and transport of CDV (uptake and efflux). A different pattern of CDV-resistance acquisition was observed between HPV⁺ and HPV⁻ tumor cell lines.

Poster Session I

4:30 PM – 6:30 PM

FERNANDES

22

Study of γ -substituted nucleoside triphosphate analogues as potential nucleosidic phosphoantigens

Javier Alguacil¹, Eric Champagne², Christian Perigaud¹, Suzanne Peyrottes¹

¹UMR5247 CNRS-University Montpellier, Montpellier, France, ²Centre de Physiopathologie de Toulouse Purpan, Toulouse, France

The role played by nucleoside 5'-triphosphates in a wide range of biological processes does not need to be further emphasized. However, their potential contribution to the immune response is much less known. In contrast to T cells that express the more prevalent $\alpha\beta$ T cell receptor and respond to peptidic antigens, T cells that express the V γ 9 δ 2 T cell receptor detect and respond to phosphorous-containing small molecules known as phosphoantigens (PAGs).¹ Because $\gamma\delta$ T cells are early responders to infections and malignancies, it has been suggested that stimulation of their activity with such PAGs may hold promise for therapeutic interventions. We have previously reported that conjugates of direct PAGs with a nucleosidic moiety also exhibited such a kind of properties.²

In the course of our research program dedicated to the study of nucleosidic phosphoantigens (NuPAGs), we aim to develop an efficient synthesis of the corresponding γ -substituted triphosphorylated nucleoside analogues. Thus, we investigated the various synthetic routes already described in the literature using ATP as starting material.³ However, the desired derivatives were isolated in poor yields due to low conversion rate, prolonged reaction times were associated with the formation of side-products, and tedious purification steps. Such multiple purification steps led to the loss of the nucleotide derivative.

Thus, another pathway adapted from the literature⁴ was tested and gave rise to better results. A variety of ATP analogs including γ -phosphate substitution and/or replacement of the natural α,β -pyrophosphate linkage with a phosphonate P-CH₂-P moiety, have been synthesized. The characterization of their ability to act as NuPAGs and their possible role in the mechanism of γ 9 δ 2 T cells stimulation are currently under study.

¹D.F. Wiemer, A.J. Wiemer, *Biochem. Pharmacol.* (2014) 89, 301–312

²Mokerjee-Basu, et al., *J. Immunol.* (2010) 184, 6920-28 and Vantourout P. et al., *J. Immunol.* (2009) 183, 3848-57

³S. Ruy, et al., *Org. Lett.* (2003), 5, 4716

⁴J. Stepinski et al., *RNA*, (2001), 7, 1486

23

Competitive-fitness studies of drug-resistant herpes simplex 2 (HSV-2) DNA polymerase mutants

G. Andrei¹, S. Gillemot¹, F. Morfin², G. Opendakker¹, R. Snoeck¹

¹Rega Institute, KU Leuven, Leuven, Belgium, ²Hospices Civils de Lyon, Lyon, France

INTRODUCTION. An important consideration for understanding dynamics of drug-resistant viruses is how resistance mutations affect viral fitness. The gold standard for estimating the relative replication capacity of a viral strain is a dual infection competition assay. Today, no such studies have been performed in herpesviruses and up to now fitness of drug-resistant mutants has compared viral growth in mono-infections.

METHODS. We have used deep-sequencing to determine viral variants that emerged after performing competitive fitness studies with HSV-2 mutants recovered from a hematopoietic stem cell transplant recipient suffering from a primary infection with involvement of multiple body sites. Plaque purified viruses with well-characterized mutations in the viral DNA polymerase were used in this study. Human embryonic lung cell cultures were infected with two viruses [wild-type (WT) + each mutant or mutant 1 + mutant 2] mixed at a 1:1 ratio based on viral titres. Following virus adsorption, cultures were incubated with or without antivirals and they were processed on days 1 throughout 7 for deep-sequencing analysis (IlluminaTM, MiSeq) to detect viral variants.

RESULTS. The A606V, F923L and M789T DNA polymerase variants but not the T934A were recovered at a similar percentage as the WT virus when they were evaluated in competition with WT virus in absence of antivirals. However, all the different mutants were found at higher proportions than the WT virus when grown in the presence of acyclovir or foscavir but not under cidofovir. When the different mutants were grown in competition against each other, the F923L and T934A showed a clear advantage over the M789T either in the presence or absence of antivirals while the A606V showed an advantage over the F923L and M789T mutants. Interestingly, the T934A showed increased fitness compared to the F923L when they were grown without antivirals or in presence of foscavir and cidofovir but not under acyclovir.

CONCLUSION. Our results indicate that fitness of HSV drug-resistant mutants is clearly affected by the presence of WT virus and other viral mutants as well as by selective drug pressure. Therefore, the dynamics of HSV populations should be taken into account during antiviral therapy.

24 Pyrazole derivatives as novel anti-flavivirus compounds

Elena M. Atzori¹, Nicoletta Desideri¹, Rossella Fioravanti¹, Antonio Carta², Irene Briguglio², Cristina Ibba³, Ilenia Delogu³, Roberta Loddo³

¹Università di Roma, Roma, Italy, ²Università di Sassari, Sassari, Italy, ³Università di Cagliari, Cagliari, Italy

The *Flaviviridae* family represents a large group of viral pathogens that consists of three genera: *Flavivirus* [type species, yellow fever virus (YFV)], *Hepacivirus* [type species, hepatitis C virus (HCV)], and *Pestivirus* [type species, bovine virus diarrhea (BVDV)]. Despite these viruses are responsible of a wide range of severe diseases and even mortality in humans and animals, specific antiviral therapies are not available for the treatment of infections caused by viruses belonging to *Flavivirus* and *Pestivirus* genera. On the other hand an effective vaccine against YFV has been available since the late 1930s, but unfortunately its utilization is incomplete in many underdeveloped areas of the world.

By screening a library of compounds in the possession of our research group, we identified pyrazole derivatives, in particular 1,4-diphenyl-1*H*-pyrazol-3-yl)methanamines, as a new class of BVDV and YFV inhibitors. The hit compound exhibited activity in the micromolar range coupled with low cytotoxicity (CC₅₀ > 100 μM against the cell lines MDBK and BHK utilized for the *in vitro* assays) [unpublished results]. Therefore, the systematic modification of all the portions of the molecular scaffold has been planned, in order to identify more active compounds and to maintain the low cytotoxicity of the hit compounds. Potent and selective inhibitors of YFV replication were obtained by the replacement of 1-phenyl ring with 1-phenylsulfonyl moiety.

25 Synthesis of Natural Product Mimetic as Possible anti-HSV Agent

Chandalata Bal¹, Harapriya Chakravarty¹, Debprasad Chattopadhyay², Ashoke Sharon¹

¹Department of Chemistry, BIT Mesra, Ranchi, India, ²ICMR Virus Unit, ID & BG Hospital, Kolkata, India

The treatment for herpes infection is limited due to ineffective clearance of virus particles and frequent emergence of drug-resistant viruses, particularly in immunocompromised patients, pregnant women and neonates. Infections caused by viruses are one of the leading causes of morbidity and mortality globally. The new chemical entity based on natural product scaffold with multiple stereo centers gives a chance, however imposes a challenge to explore its antiviral activity due to limited exploration of its mimetic. Thus, synthesis of new molecules were initiated using two distinct combinations of imidazo isoquinoline motif with three fused ring system (like Harmaline).

In our recent studies, we found that the extract from *Ophiorrhiza nicobarica*, an ethnomedicine containing triterpene and a -carboline indole alkaloid had significant anti-HSV activities. In continuation, our natural product mimetic approach including *in-silico* modeling studies yielded discovery of new natural product based synthetic compound I. The synthesized compounds showed significant antiviral activity against HSV-1 and HSV-2. It showed CC₅₀ of 200 and 275 mM with EC₅₀ of 26 to 29.5 mM against HSV-1 and HSV-2 with SI value of 7.32-9.48. The preliminary lead optimization is in progress to discover anti-HSV clinical molecule. The mode of action studies suggest that the compounds may not interfere in viral attachment or penetration, however, reduced the expression of ICP4 and ICP27 (immediate-early gene products) as well as the HSV DNA polymerase. (Acknowledgement: DBT & DST, Government of India for financial support.)

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Synthesis and Evaluation of Octadecyloxyethyl benzyl 9-[(2-phosphonmethoxy)ethyl]guanine (ODE-Bn-PMEG), a Potent Inhibitor of HPV DNA AmplificationJames R. Beadle¹, Nadejda Valiaeva¹, Hsu-kun Wang², Guang Yang², Louise T. Chow², Karl Y. Hostetler¹¹University of California, San Diego, La Jolla, USA, ²University of Alabama at Birmingham, Birmingham, USA

Human papillomavirus (HPV) high-risk genotypes are the primary cause of anogenital tract carcinomas, including cervical cancer, the second most common malignancy in women worldwide. Current therapies for HPV-related pre-cancerous lesions rely on their surgical removal, cryotherapy, or destruction with cytotoxic agents. Development of an HPV-specific topical antiviral therapy could provide a simpler, more accessible treatment option. Acyclic nucleoside phosphonates (ANPs) are known to have broad spectrum antiviral activity, including efficacy against papillomaviruses. We synthesized a new series of alkoxyalkyl ANP diesters and screened them for in vitro anti-HPV activity. For example, octadecyloxyethyl 9-[(2-phosphonmethoxy)ethyl]guanine (ODE-PMEG) was prepared as previously described. The monoester was then treated with benzyl alcohol and the condensation reagent PyBOP (diisopropylethylamine, N,N-DMF), generating the diester ODE-benzyl-PMEG as a racemic mixture. The Rp and Sp enantiomers were obtained by using preparative HPLC and a chiral stationary phase (Phenomenex Lux™ Cellulose 1). We observed a potent inhibition of transient amplification of HPV-11 and HPV-16 replication origin-containing plasmids in HEK-293 cells when co-transfected with expression vectors of HPV-11 and -16 E1 and E2 replication proteins. For the racemate, EC₅₀s for the HPV-11 and HPV-16 genotypes were determined to be 0.32 μM and 0.20 μM, respectively, with selectivity indices >625. Similar activity was noted for the separated enantiomers. The compound was also shown to inhibit HPV-18 genomic DNA amplification in organotypic cultures of productively infected primary human keratinocytes (EC₅₀ = 0.33 μM). In summary, ODE-Bn-PMEG is a potent anti-HPV compound which could fill an unmet need as a non-surgical treatment option for HPV-positive pre-cancerous lesions.

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Correlation between Side Arm Dipole Moments and Human CD4 Down-Modulating Abilities in Unsymmetrical Cyclotriazadisulfonamide (CADA) Compounds with Anti-HIV ActivitiesThomas W. Bell¹, Reena Chawla¹, Victor van Puyenbroeck², Dominique Schols², Kurt Vermeire²¹Department of Chemistry, University of Nevada, Reno, USA, ²Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Cyclotriazadisulfonamide (CADA) compounds inhibit HIV entry and virus replication in cell culture by preventing surface expression of human CD4, the main cellular receptor for HIV. The target of CADA has recently been identified as the N-terminal signal peptide of nascent CD4 (Vermeire *et al.*, *PLoS Biology*, 2014, 12(12):e1002011. DOI: 10.1371/journal.pbio.1002011). Through selective binding to the CD4 signal peptide, CADA inhibits co-translational translocation of human CD4 across the membrane of the endoplasmic reticulum. Here, a series of 35 new, unsymmetrical CADA analogs have been synthesized and evaluated for CD4 down-modulation and anti-HIV potency. CK147, having a cyclohexylmethyl tail group and one 4-*N,N*-dimethylaminobenzenesulfonamide side arm, was found to have highest potency towards CD4 down-modulation in CHO cells transfected with a fluorescent CD4 fusion protein (IC₅₀ = 44 nM) and in the T-lymphoid cell line MT-4 expressing human CD4 naturally (IC₅₀ = 100 nM). In addition, the CK147-induced reduction in CD4 correlated with enhanced anti-HIV-1 NL4.3 activity (IC₅₀ = 180 nM). The most potent compounds have one or more electron-donating groups in the para or meta positions of one of the two benzenesulfonamide side arms and also lack hydrogen bond donors. We calculated the dipole moments of *N,N*-dimethylbenzenesulfonamides modeling the side arms of 17 CADA compounds with benzyl tail groups, and no hydrogen bond donors, and found a significant correlation (R² = 0.63) with CD4 down-modulation potency. We postulate that a dipole-dipole interaction between this side arm and the CD4 signal peptide strongly contributes to binding and inhibition of co-translational translocation.

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Effects of nonnucleoside HIV-1 reverse transcriptase inhibitors on the initiation of (+)-strand DNA synthesis

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HIV-1 reverse transcriptase (RT) converts viral genomic RNA into double-stranded DNA. The RT uses the viral RNA as template to synthesize a (–)-strand DNA chain. Then, this product serves as template to obtain the (+)-strand DNA. Polypurine tracts (PPTs) resilient to RNase H cleavage are used as primers during (+)-strand DNA synthesis. Premature PPT degradation impairs reverse transcription. Previous studies have shown that nonnucleoside RT inhibitors (NNRTIs) such as nevirapine and efavirenz facilitate PPT removal. However, N348I in the RT connection subdomain could attenuate the effects of nevirapine. We have studied how recently approved NNRTIs (etravirine and rilpivirine) and RT connection subdomain mutations (N348I, T369I, T369V and T376S) affect initiation of (+)-strand DNA synthesis.

Drug susceptibility assays were carried out in MT-4 cells with recombinant HIV-1 bearing wild-type (WT) or mutant RTs (i.e. with substitutions in the connection subdomain and/or the NNRTI binding site). WT and mutant RTs were obtained as p66/p51 heterodimers. DNA polymerization and RNase H activity assays were carried out with template-primer hybrids with intact or elongated PPT sequences [UUAAAAGAAAAGGGGGG (17 rNTPs) and UUAAAAGAAAAGGGGGGactggaag (17 rNTPs + 8 dNTPs; PPT17r8d)].

All NNRTIs enhanced PPT17r8d cleavage at the RNA-DNA junction but rilpivirine had the lowest stimulatory effect. Cleavage by the WT RT was not affected by mutations T369I, T369V or T376S. However, in the absence or presence of nevirapine or efavirenz, N348I and N348I/T369I RTs showed reduced PPT cleavage. Those mutants had an altered RNase H cleavage window, as demonstrated with PPT-containing RNA/DNA hybrids of different lengths. RNase H-mediated trimming of the 3'-end of the 17-nt PPT primer was detected with all tested NNRTIs, but higher degradation was observed with rilpivirine and efavirenz. We found that rilpivirine was the most potent inhibitor in PPT primer extension assays, since it inhibits nucleotide incorporation while preventing the use of available PPTs. Those inhibitory effects were attenuated in the presence of the rilpivirine resistance mutation E138K.

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Activity of Antiviral compounds in cells model of virus co-infection

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INTRODUCTION: The problem of modern medicine is a high spread of infections caused by herpes virus and adenovirus and the tendency of mixed infections of patients. Search of drugs with relatively high activity against co-associated viruses by inhibiting their reproduction and transmission is an important task. Activity of antiviral drugs under condition of co-infection is not studied sufficiently. Adenovirus and herpes simplex virus belong to the widespread viruses and are capable to prolonged storage in the body in latent form.

METHODS: Cytotoxic activity of the drugs was studied by MTT-assay. Model of co-infection of MDBK cells created in our laboratory was used to research the efficiency of antiviral agents of broad action spectrum (ganciclovir and ribavirin) against human adenovirus serotype 5 (*HAdV5*) and herpes simplex virus 1 type (*HSV1/US*). Antiviral effect of drugs was studied using a medical scheme of introduction of preparation (as component of a supporting medium). Analysis of virus DNA synthesis in the infected cells after treatment with drugs was carried out with RT-qPCR. The effectiveness of preparations under mono- and mixed infections was studied by plaque reduction method.

RESULTS: Use of the drug ribavirin in conditions of mono infection led to inhibition of DNA of replication of adenovirus by 41% and herpes virus by 29%. Ribavirin was ineffective against *H dV5* and its activity against herpes virus remained unchanged under conditions of co-infection of cells. Use of the drug in these conditions led to an increased titer of adenovirus in 2.9 times. The analysis of antiviral activity of drug ganciclovir on models of mono infections showed reduction of reproduction of *HSV-1/US* by 60% and *HAdV-5* by 4%. During the co-infection of cells the ganciclovir inhibited the reproduction of herpes virus by 61%. Effectiveness of the drug against adenovirus increased 20%.

CONCLUSIONS: Thus, the difference of drugs activity against co-associated viruses in conditions of mono and mixed infections of cells was shown. An increase as well as inhibition of drugs activity were detected, this may result in the formation of resistant strains of viruses.

31 **New Approaches In Detecting Different Causative Agents and Prediction of Disease Progression**

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The purpose of research is to study a possibility of detection of presence of pathogens in the infected objects with “ SC-BARS”[1] on the basis of new approaches to the fuzzy variables processing under conditions of object’s noise.

The next tasks were set: To develop a new algorithm of nosological markers data processing, which were recorded from

hardware and software complex of spectral correction « SC-BARS”, with the use of Fuzzy-technology processing [3], with the purpose of recognition of different types of pathogens in the infected objects.

To study a possibility of application of the developed data processing algorithm for detecting the pathogen in the studied object, type of pathogen and degree of object infection.

The article presents the general formal task definition of diagnosing pathogens, the approach to solving the task and a number of obtained results with using the given approach.

32 **Optimizing HTS Assays to Screen for Antiviral Agents**

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The HTS Center at Southern Research serves as the screening core for the Antiviral Drug Discovery and Development Center (AD3C) funded by the NIH via a CETR (U19) program grant. As such, the SR HTS Center is responsible for developing, optimizing and conducting HTS assays to identify active compounds to serve as potential starting points in the discovery and development of novel antiviral agents targeting flaviviruses, coronaviruses, alphaviruses and influenza. By screening a common collection of 350,000 compounds against each virus, we hope to identify agents with broad spectrum activity. To achieve necessary performance characteristics for HTS, assay optimization requires attention to parameters unique to antiviral assays. As an example, we show that the preparation and source of virus stock, as well as the choice of host cell, are important parameters that affect performance of cytopathic effect (CPE) assays. Although the CPE assay is widely used in HTS, it is not always the best choice. We will describe cell-based assays using different endpoint measures amenable to HTS with more physiological relevance than CPE. The first of these assays uses a reporter readout measuring induced firefly luciferase activity. The second assay employs laser scanning cytometry to measure the surface expression of viral protein on the infected host cell. A third assay uses qPCR to directly measure virus titer. Comparative optimization and performance data for each of these assays will be presented along with a discussion of the pros and cons for the different assay formats.

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Design, synthesis and biological evaluation of human DDX3 inhibitors with multiple antiviral activities

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The cellular helicase DEAD-box 3 (DDX3) is known to be an essential host factor for major human viral pathogens such as HIV-1 and Hepatitis B and C viruses as well as for the replication of viral agents responsible for orphan diseases such as Dengue virus (DENV), West-Nile virus (WNV), Human T-cell leukemia Virus (HTLV)-1 and Japanese Encephalitis Virus (JEV). No specific and effective pharmacological treatment is currently available for these latter pathogens, while it is becoming clear that they pose an increasing threat to EU citizens and may eventually lead to sustained epidemics in Europe. All compounds that are currently approved for the treatment of viral infections target viral proteins. Since viruses have evolved the capacity to exploit the cell's molecular machineries as essential components of their replicative cycle, agents designed to interrupt viral replication could, in principle, target with equal effectiveness either a viral or cellular polypeptide. In the case of a unique viral function, the Achilles' heel of such an approach is viral resistance to the drugs. The alternative approach, targeting a cellular factor that is required for viral replication, should help to overcome the problem of viral resistance. Theoretically, a drug targeting a cellular factor could also inhibit all viruses that are dependent on the same host factor. Recently, it has been revealed that the cellular ATPase/RNA helicase X-linked DEAD-box polypeptide 3 (DDX3) is an essential host factor for the replication of several viruses.¹ Accordingly, our research group is working in targeting both the ATPase and RNA binding regions of DDX3.²⁻⁵ Most of the synthesized derivatives were able to inhibit the DDX3 helicase activity at submicromolar concentration. Furthermore, these compounds showed anti-HCV and anti-HIV activity in cells, as well as a good inhibitory activity against JEV, DENV and WNV infections. No cytotoxicity was found for the studied compounds up to 20 μ M concentration.

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Inhibitory Effect of Cidofovir on Parvovirus B19 Replication

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Parvovirus B19 (B19V) is a human ssDNA virus responsible for a wide range of clinical manifestations, still lacking for a specific antiviral therapy. The identification of compounds active against B19V may add therapeutic options to the treatment of B19V infections. To this purpose, focus was raised to cidofovir (CDV), an acyclic nucleoside phosphonate broadly active against dsDNA viruses.

Two model systems were used to assess the inhibitory effect of CDV on B19V replication: 1) the UT7/EpoS1 megakaryoblastoid cell line, able to support B19V DNA replication; 2) the ex vivo expanded CD36+ erythroid progenitor cells (EPCs), a highly permissive system for B19V replication and expression. EPCs were generated from peripheral blood, via culture in a medium containing erythropoietic growth factors (EPO, SCF and IL-3), for up to 18 days. Expression of EPC differentiation markers ranged from a minimum of 10% at 3-day to a 80% at 15-day culturing. Infection at different stages indicated that the EPC system was fully permissive to B19V between days 6 and 15 of EPCs in vitro growth and differentiation.

Experiments were carried out at different multiplicity of infection ($10^0 - 10^4$ genomes/cell) and CDV concentrations (0.1 – 500 μ M). The effects of CDV were evaluated by its capacity to inhibit viral nucleic acid synthesis, as determined by means of q PCR assays for quantification of viral nucleic acids. CDV showed a dose-dependent inhibiting activity on B19V replication within infected UT7/EpoS1, allowing for the determination of EC₅₀ and EC₉₀ values (7.45–41.27 μ M, and 84.73–360.7 μ M, respectively). In EPCs, a significant reduction on B19V DNA amounts was obtained only at 500 μ M (68.2–92.8%). However, cell-culture supernatants from B19V-infected, CDV-treated EPCs were used for serial infection of EPCs in the presence of CDV, leading to a progressive inhibition of B19V replication compared to untreated controls.

With regard to the host cells, the drug did not interfere with the overall cellular DNA replication and metabolic activity. The effect of CDV on B19V could be likely related to a specific inhibition on the viral replication process, indicating the possibility of developing an antiviral strategy against a relevant human pathogenic virus.

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Structure-Based Identification and Chemical Optimization of Anti-Hcv Pyrazolobenzothiazine Derivatives Targeting NS5B

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Hepatitis C virus (HCV) is a small positive-sense single-stranded RNA virus which chronically infects 170-200 million people worldwide leading to severe liver diseases.¹ Current anti-HCV treatments based on direct-acting antivirals specifically targeting viral proteins have significantly improved the therapy in comparison to traditional interferon and ribavirin combination.² Our active HCV drug discovery program is mainly focused on inhibition of HCV replication targeting NS5B polymerase.³⁻⁵ In this communication, the discovery of the pyrazolobenzothiazine scaffold as a promising chemotype able to bind NS5B palm site I and inhibit HCV replication is described. In particular, a structure-based virtual screening of our in-house library followed by rational chemical optimization, organic synthesis, biochemical, and biological testing led to low micromolar NS5B inhibitors endowed with a promising and not toxic anti-HCV activity in cellular assays.

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N-[4-(1H(2H) benzotriazol-1(2)-yl)phenyl]alkylcarboxamides: a new class of antiviral agents

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The broad experience on chemical and biological behavior of benzotriazole derivatives gained by our research group led us to synthesize and biologically evaluate a series of N-[4-(1H(2H)-benzotriazol-1(2)-yl)phenyl]alkylcarboxamides and N,N'-bis[4-(1H(2H)-benzotriazol-1(2)-yl)phenyl]alkyldicarboxamides against viruses representative of Picornaviridae (CVB-2 and Sb-1), Paramyxoviridae (RSV) and Flaviviridae (BVDV and YFV) families.

Particularly, viruses belonging to Flaviviridae (ssRNA+) and Paramyxoviridae (ssRNA-) families continue to pose threats to public health. HCV (Flavivirus, belonging to Hepaciviruses genus) is the leading cause of cirrhosis, end-stage liver disease, and liver cancer. It is responsible for thousands of deaths per year in the United States and no vaccine is available to prevent the infection. Just some new drugs arrived to the market and more are actually in study. RSV (Paramyxoviridae, belonging to Pneumovirus genus) is a leading cause of severe lower respiratory tract infections (bronchiolitis) and pneumonia in children younger than 1 year of age and in older adults. At present, neither specific antiviral therapy nor vaccine is available for the treatment.

All compounds showed from acceptable to good cytotoxicity profile in MT-4 cells, diverse antiviral activities and for some of them selectivity against CVB-2 was highlighted.

With the aim to enhance the anti-Flaviviridae activity we report the synthesis and biological evaluation of a new series of monomeric and dimeric analogues bearing on the benzotriazole moiety one or two chlorine atoms at position 5 and 6. All of them have been tested on the previously analyzed virus panel and some of them emerged for their potent activity in the low micromolar range against BVDV, classically used as a surrogate model of HCV, and RSV.

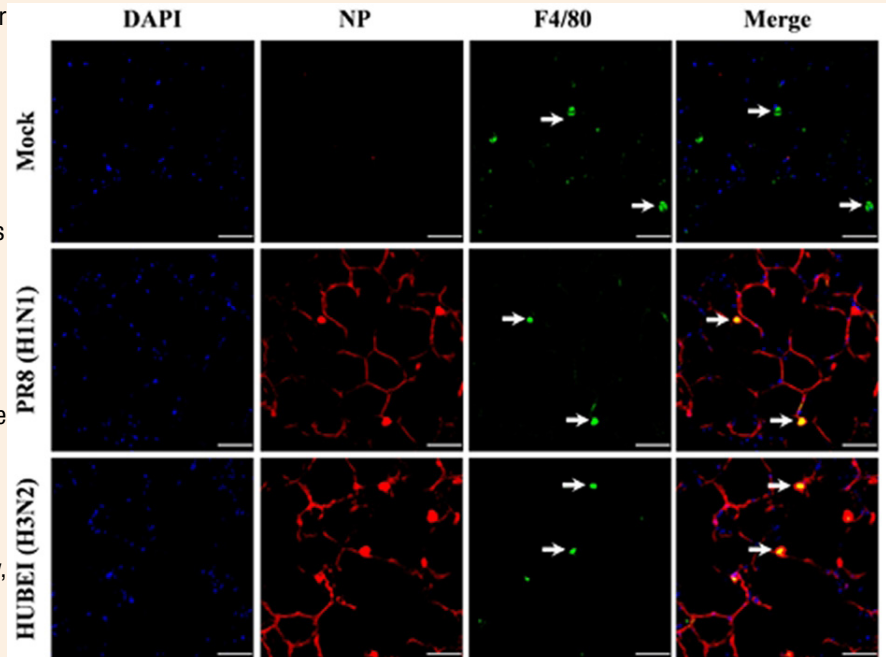
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Mouse lung slices: an ex vivo model for evaluation of antiviral and anti-inflammatory agents against influenza viruses

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The influenza A virus can cause recur within 48 hours of symptom onset. Research on anti-inflammatory therapy to ameliorate influenza induced inflammation is currently underway and seems important to impact the clinical outcome. Both antiviral and anti-inflammatory drugs with novel mechanisms of action are urgently needed. Current methods for the evaluation of efficacy of anti-influenza drugs rely mostly on transformed cells and animals. Transformed cell models are distantly related to physiological and pathological conditions. Though animals are the best choices for preclinical drug testing, they are not time- and cost-efficient. In this study, we established an *ex vivo* model using mouse lung slices to evaluate both antiviral and anti-inflammatory



agents against influenza virus infection. Influenza viruses can replicate efficiently in the mouse lung slices and trigger significant cytokine and chemokine responses, which correlate positively to that *in vivo*. Furthermore, a set of agents with known antiviral or (and) anti-inflammatory were tested for antiviral and anti-inflammation activities to validate the *ex vivo* model. Our results suggest that mouse lung slices provide a robust, convenient and cost-efficient model for the assessment of both antiviral and anti-inflammatory agents against influenza virus infection in one assay. This *ex vivo* model may predict efficacy of drug candidates in antiviral and anti-inflammation activities *in vivo*.

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Molecular epidemiology of emerging Crimean Congo Haemorrhagic Fever virus in Iran

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BACKGROUND: Crimean Congo Haemorrhagic Fever (CCHF) is a zoonotic viral infection which caused by CCHF virus, belongs to *Nairovirus* genus and Bunyaviridae family. The initial phylogeny study on CCHFV in Iran showed that isolated Iranian strain (ArTeh193-3) was similar to Senegalese strain.

METHODS: The viral RNA was extracted from human sera samples between 2004 and 2014. The virus genome was examined by RT-PCR. PCR products were sequenced using Big Dye Terminator V3.1 Cycle sequencing Kit with Modified Sanger Sequencing Method by ABI Genetic Analyzer 3130. The sequence alignment was performed by ClustalW and a scaled phylogenetic tree generated by the Maximum Likelihood (ML) with Kimura 2-parameter distance using Mega5 software.

RESULTS: Our earlier phylogenetic analysis of partial S-segment nucleotide sequences in Iran, in 2004, illustrated that the CCHFV isolates were clustered with strains from Pakistan. These data also demonstrated that the Iranian examined strains and the previously published CCHFV strain ArTeh193-3 clustered into different genetic groups, indicating that at least two genetic lineages of CCHFV could be co-circulating in Iran. Further investigations demonstrated that a variant was clustered with the Iraqi strain. A

recent phylogenetic study on the CCHFV genome sequences from the North of Iran showed similarity between isolated strains and Russian strains.

CONCLUSION: In our previous studies, CCHF genome S-segment sequences were genetically characterized and results illustrated a close relation with Matin strain (Pakistani Strain), whereas, our study in 2008 revealed the circulation of Iraqi strain in Iran. To date, according to all previous phylogenetic studies, it can be claimed that Senegalese, Pakistani, Iraqi and Russian strains of CCHFV are known to be circulating in Iran.

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Characterization of the Dynamics of HBV Resistances and Genetic Diversity from Longitudinally Antiviral Therapy Treated Patients with Next Generation Sequencing

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HBV drug resistance testing is routinely performed prior to antiretroviral treatment. Long durations of therapy are required and often lead to the emergence of drug resistance. While population sequencing detects variants with frequencies above 15–20% of the viral quasispecies, deep sequencing strategies allow for sensitivities of 1% and below. Next-generation sequencing (NGS) technologies allow the rapid and cost-effective acquisition of thousands to millions of short DNA sequences from a single sample. Here, we presented data of the clinical samples to obtain insights in sensitivity, accuracy and the putative impact of minority variants on resistance interpretation. The thirty two specimens from 8 patients on polymerase were performed from specimen for whom resistance testing was longitudinally collected for about 10 years. For deep sequencing, the PCR products were used for Nextera XT® library preparation and sequenced using Illumina's HiSeq 2500 sequencing system. Bioinformatics analyses were performed using an automated customized pipeline. The dynamics of lamivudine, adefovir-resistant variants were complex and differed among patients as results of evolving differences in variant fitness. NGS analysis revealed successive waves of selection of HBV population with single and multiple amino acid substitution. In addition to, G-to-A hypermutation mediated by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like family to cytidine deaminases was estimated to be present in 0.6% of polymerase gene. NGS detected low-prevalence HBV variants with lamivudine, adefovir, entecavir, G-to-A hypermutation with a sensitivity not previously possible. Substitutions conferring HBV resistances to antiviral agents exist as a passage of treatments are much more complex and heterogeneous than previously thought and thus far unknown amino acid substitutions.

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Phenotypic characterization of HIV-1 reverse transcriptase associated activities of a multiresistant subtype AG strain

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Highly active antiretroviral therapy (HAART) against human immunodeficiency virus (HIV) is often hampered by emergence of drug-resistant viruses. We characterized a HIV-1 reverse transcriptase (RT) subcloned from a multiresistant (MR) HIV-1 subtype CRF02_AG strain, isolated from a patient treated by different HAART combinations, harboring 43 amino acid substitutions conferring resistance to various non-nucleoside and nucleoside RT inhibitors such AZT. It is well known that AZT resistance can be achieved by: (i) excision of the incorporated AZT monophosphate and (ii) discrimination between AZTTP and TTP during nucleotide incorporation. Interestingly, MR RT contains 4 of the 5 substitutions (M41L, D67N, T215Y and K219E) known as important for AZTMP excision, as well as 4 out of 5 substitutions (A62V, V75I, F116Y and Q151M) enabling AZT discrimination. To determine which AZT resistance mechanism in MR RT is still functional and if the two mechanisms are mutually exclusive, we tested both mechanisms in MR RT and in a number MR RTs obtained by inserting the substitution K70R and reverting to wt R65 and M151. All MR RTs were still able to discriminate AZTTP, while only upon further introduction of R65K (MR-K70R-M151Q-R65K) AZTMP excision could be observed and discrimination was abolished, indicating that discrimination is favored over excision in the MR RT. Furthermore, MR RT harbors several amino acid substitutions in the Ribonuclease H (RNase H) domain, with only one (K512R) previously related to thymidine analogue mutations. Since the relevance of the other substitutions related to resistance could be also crucial for treatment of patients, we asked if RNase H function was altered in the MR RTs and if wt and subtype AG MR RTs were still sensitive to known RNase H inhibitors. Data showed that all MR RTs have a well preserved RNase H function and that they have similar susceptibility towards a variety of RNase H inhibitors, overall suggesting the importance of developing RNase H inhibitors against HIV multidrug resistance.

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Structural Optimization of the Cycloheptathiophene-3-carboxamide scaffold to target HIV-1 Ribonuclease H

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Despite the significant progress achieved with the Combination Antiretroviral Therapy in the fight against the HIV infection, the difficulty to completely eradicate the virus together with the rapid emergence of multi-drug-resistant virus strains clearly underline the need to search for innovative agents, possibly endowed with novel mechanism of actions. In this contest, due to its pivotal role in HIV replication, the reverse transcriptase (RT)-associated ribonuclease H (RNase H) function has been elected as a promising target. While no drug is yet in clinical trials, only a limited number of RNase H inhibitors have been reported to date mainly targeting the RNase H active site.¹ Based on the me-too strategy, an in-house cycloheptathiophene-3-carboxamide library has been screened identifying a promising compound that showed IC₅₀ of 4.72 ± 0.044 μM, comparable to those reported for other RNase H allosteric inhibitors.¹

Starting from this hit compound, a structural optimization was attempted synthesizing an enlarged series of cycloheptathiophene-3-carboxamide analogues.

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Ester Prodrugs for the Delivery of Anti-Filoviral N-Alkyldeoxynojirimycin IHVR-19029

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Ebola and Marburg viruses in the flaviviridae family cause hemorrhagic fever with high lethality in humans. Currently, there are neither effective vaccines nor antiviral interventions to manage either of the diseases. These viruses present similar clinical symptoms, so a drug that is safe and effective against both Ebola and Marburg would be an enormous benefit. We have discovered a novel class of N-alkyldeoxynojirimycins (NADNJs), targeting host endoplasmic reticulum (ER) -glucosidases I and II, with broad-spectrum antiviral activity against multiple enveloped viruses in vitro. Specifically, a representative lead compound, IHVR-19029, demonstrated significant protection in the mouse models of Marburg and Ebola virus infection, when administered through injection. However, there are concerns regarding IHVR-19029. It exhibited a short half-life and low oral bioavailability in mice. When delivered orally, it may also trigger GI distress, due to nonspecific inhibition of carbohydrate metabolizing gut glucosidases. Here, we report our prodrug strategy to increase oral bioavailability and avoid off-target inhibition of intestinal glucosidases. We have synthesized ester prodrugs and evaluated their activities in cell-based and ER-glucosidase enzymatic assays. PK profiling is currently underway.

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Nonclinical Pharmacokinetics and Preferential Respiratory Tract Distribution of GS-5806, Fusion Inhibitor of Respiratory Syncytial Virus

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GS-5806 is an inhibitor of human respiratory syncytial virus with potent antiviral activity against a broad range of subtype A and B clinical isolates and in animal models of infection. As part of preclinical development, the pharmacokinetics of GS-5806 in rats, dogs, cynomolgus and rhesus monkeys, and bovines was determined and its tissue distribution into major organs and the respiratory tract was evaluated in rats. Additionally, the distribution of GS-5806 into epithelial lining fluid (ELF) and lung tissues was assessed in the rats and bovines. There were no major interspecies differences in GS-5806 pharmacokinetics. Following intravenous administration, the elimination half-life of GS-5806 ranged from 3 h in rats to 9 h in bovines. The total volume of distribution (3 – 10 L/kg) exceeded the volume of total body water in all species, indicating extensive tissue distribution. Systemic clearance of GS-5806 was lower than the hepatic blood flow in all species. Oral bioavailability of GS-5806 ranged from 46 to 100%.

[¹⁴C] GS-5806 related radioactivity in the rat upper and lower respiratory tract tissues significantly exceeded the systemic levels. GS-5806 demonstrated significant penetration into rat and bovine ELF (19 – 12 fold over plasma levels) and lung tissues (87 – 313 fold). The preferential distribution into ELF and lung was considerably greater for GS-5806 than for its structural analog GS-577855 lacking the primary amine group. These studies demonstrate that GS-5806 has favorable pharmacokinetics in all species and therapeutic levels in target organs are achievable and expected to be maintained for prolonged periods following a QD dose.

45 Pharmacokinetics and Preclinical Characterization of GS-5806, Oral Respiratory Syncytial Virus Inhibitor, in Adult and Neonate Preclinical Species

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GS-5806 is an inhibitor of human respiratory syncytial virus with potent antiviral activity against a broad range of clinical isolates. It was found to be efficacious in animal models of infection and in human challenge studies. In vitro metabolism of GS-5806 was assessed in hepatocytes from different species and the pharmacokinetics and metabolic profiles were determined in adult and neonate rats and dogs. The distribution of GS-5806 into the respiratory tract was also evaluated in selected species.

The major metabolic pathways for GS-5806 in hepatocytes included oxidative deamination in human, monkey, and dog hepatocytes and Nacetylation on the aminopyrrolidine ring in the human, rat, and cynomolgus monkey hepatocytes. There were no major interspecies differences in GS-5806 pharmacokinetics. The $T_{1/2}$ of GS-5806 ranged from 3 to 10 h. The volume of distribution exceeded the volume of total body water, indicating extensive tissue distribution. Systemic clearance was significantly lower than hepatic blood flow. Oral bioavailability of GS-5806 ranged from 46% to 100%. There were no major differences in GS-5806 pharmacokinetics and metabolic profiles between adult and young animals. From the in vitro predicted human hepatic clearance and volume of distribution estimated allometrically, the $T_{1/2}$ in both adults and infants was projected to be suitable for the QD dosing regimen.

GS-5806 demonstrated significant penetration into rat and bovine lung tissues (87-313 fold over plasma levels) and epithelial lining fluid (19-12 fold). The preferential distribution into the respiratory tract was considerably greater for GS-5806 than for its structural analog GS-557855 lacking the primary amine group.

These studies demonstrate that GS-5806 has favorable pharmacokinetics in preclinical species achieving high levels in the respiratory tract and that its unique structural elements are responsible for the preferential lung distribution. The studies also demonstrate that there are no major differences in GS-5806 pharmacokinetics or distribution between adult and young animals.

46 The Antiviral Drug Discovery and Development Center (AD3C): an Academic Drug Discovery Consortium for (Re-)Emerging Viral Infections

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The Antiviral Drug Discovery and Development Center (AD3C), coordinated out of the University of Alabama at Birmingham (UAB) has, at its center, the theme to develop new small molecule therapeutics for emerging and re-emerging viral infections. AD3C focuses on developing drugs for four virus families: influenza, flaviviruses, coronaviruses and alphaviruses— infections causing diseases including West Nile virus, SARS, chikungunya and dengue viruses. Researchers work to target and inhibit enzymes essential for viral replication, with AD3C providing an infrastructure to accelerate the development of new potential drugs from the lab towards the clinic. UAB is collaborating with top virologists across the USA who are already working with these agents, at institutions that include Oregon Health and Science University, Washington University, Vanderbilt University, the University of North Carolina (UNC) at Chapel Hill and Southern Research, also located in Birmingham, AL. The families of viruses targeted within AD3C represent both biologic threats and unmet medical needs. The global burden of these diseases is enormous, with West Nile virus and influenza routinely infecting citizens around the world. AD3C will also strive to develop therapies for emerging infections such as coronaviruses, dengue and chikungunya.

The research focuses on the inhibition of viral replication, especially viral polymerases. The participating researchers are focusing on target validation, high throughput screening to identify novel chemical scaffolds, and basic virologic research to prove and further probe the exact mechanism of action of identified lead molecules in viral replication. Medicinal chemistry and lead development activities at SR will advance identified compounds down the drug discovery and development pathway, ultimately leading to preclinical evaluation of promising new drug candidates to treat these diseases.

AD3C is a model for how academic drug discovery, done with the right expertise and the appropriate advanced research collaborators, can move swiftly and efficiently to develop new drugs for re(emerging) infections

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New thiosemicarbazones derived from 1-indanones with antiviral activity against bovine viral diarrhea virus.

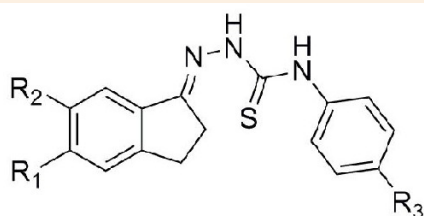
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Bovine viral diarrhea virus (BVDV) is the prototype *Pestivirus*. BVDV infection is distributed worldwide and causes serious problems for the livestock industry. The thiosemicarbazone (TSC) of 5,6-dimethoxy-1-indanone (lead compound) is a potent non-nucleoside polymerase inhibitor of BVDV.

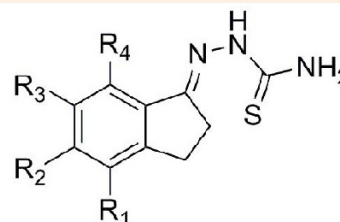
In order to find compounds with an improved anti-BVDV activity, a series of thirteen novel TSCs was synthesized. The aim of this work was to evaluate their antiviral activity *in vitro* against BVDV and to preliminarily analyze their structure-activity relationship. The most active compounds were also evaluated against BVDV mutants resistant to the lead compound (BVDV-TSC^r).

From the *N*⁴-substituted TSCs with phenyl groups (1-9), only derivatives 4-9 were active, suggesting that the 5,6-dimethoxy substitution would improve the anti-BVDV activity. It should be noted that the nature of the substituent at the R₃ position has an important effect: compounds with electron-withdrawing substituents (7 and 8) were the most active. Derivative 8 (R₃=NO₂) resulted to be almost six times more active than the lead compound (EC₅₀= 0.7±0.3 and 3.8±0.4 μM, respectively). Among the TSCs 10-13, only compound 11 exerted an activity similar to that of the lead compound, suggesting that the NO₂ group at the R₃ position would enhance the antiviral activity in the absence of the 5,6-dimethoxy substitution. Finally, BVDV-TSC^r were also resistant to compound 8 but, interestingly, they were slightly inhibited by compound 11. Further experiments and docking studies of these compounds should be held to determine the molecular interactions with the viral polymerase.



Thiosemicarbazones *N*⁴-aryl-substituted derived from 1-indanones (*N*⁴-TSCs)

- 1 R₁=R₂=R₃= H
- 2 R₁=R₂= H, R₃= CH₃
- 3 R₁=R₂= H, R₃= Cl
- 4 R₁=R₂= OCH₃, R₃= H
- 5 R₁=R₂= OCH₃, R₃= CH₃
- 6 R₁=R₂= OCH₃, R₃= Cl
- 7 R₁=R₂= OCH₃, R₃= F
- 8 R₁=R₂= OCH₃, R₃= NO₂
- 9 R₁=R₂= OCH₃, R₃= OCH₃



Thiosemicarbazones derived from 1-indanones (TSCs)

- 10 R₁=NO₂, R₂=R₃=R₄= H
- 11 R₁=R₂=R₄= H, R₃= NO₂
- 12 R₁=R₃=R₄= H, R₂= F
- 13 R₁=R₄= CH₃, R₂= Br, R₃= H

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Computer-aided discovery of small-molecules against norovirus**Salvatore Ferla¹**, Marcella Bassetto¹, Sebastiano Ferla^{1,2}, Salvatore Guccione², Johan Neyts³, Joana Rocha-Pereira³, Pieter Leyssen³, Andrea Brancale¹¹Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff, United Kingdom, ²Dipartimento di Scienze del Farmaco, Università degli Studi di Catania, Catania, Italy, ³Rega Institute for Medical Research, KU Leuven, Belgium

Noroviruses are small non-enveloped positive-sense single-stranded RNA viruses that belong to the *Caliciviridae* family. Noroviruses are the first cause of foodborne illness around the world, causing extensive outbreaks of acute gastroenteritis. In developing countries, around 200,000 children die annually due to diarrhea caused by norovirus infection. Norovirus outbreaks are a major culprit for the closure of hospital wards. Currently, no vaccines or antivirals are available to prevent or cure norovirus infection, being the treatment limited to electrolyte replenishment for the dehydrated patients.

In the last years, several human norovirus proteins have been functionally and structurally characterized, opening a new scenario in the development of a potential antiviral treatment. Among all the viral proteins, we decided to focus our studies on two main targets, which have been reported to be fundamental for the virus life cycle: the P domain of the viral capsid, which interacts with individual oligosaccharide residues of human histo-blood antigens (HBGAs), favoring the initial viral attachment to the host cell, and the NS7-RNA-dependent RNA polymerase (RdRp), which plays a key role in genome replication and in the synthesis and amplification of the additional subgenomic RNA.

Different computer-aided techniques were employed to identify potential novel small-molecules able to interfere with the P domain-HBGAs binding in the first case, and to inhibit the RdRp activity in the second. A structure-based virtual screening of commercially available drug-like compounds (~300,000) was performed for each protein target, whereas a shape-comparison screening of small molecule libraries was carried only for the RdRp.

These studies led to a final selection of 34 compounds (9 for the P domain-HBGAs binding interaction and 25 for the RdRp inhibition), which were biologically evaluated in order to verify their potential as inhibitors of the norovirus life cycle.

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Development of a high-throughput screening assay for alphavirus RNA capping inhibitors**Brian J. Geiss**

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Alphaviruses are small RNA viruses that are a serious threat to global public health. Chikungunya (CHIKV) and Venezuelan Equine Encephalitis (VEEV) viruses are alphaviruses of particular concern. CHIKV causes debilitating chronic joint pain in infected patients. Since 2007 CHIKV has spread from India, through Africa and Europe, and has recently emerged in the Western Hemisphere resulting in upwards of 750,000 cases in the Caribbean and Central/South Americas a single year. VEEV was developed as a biological weapon by several countries due to the severe encephalitic disease it causes that results in mortality rates upwards of 80%. The mosquitoes that transmit VEEV and CHIKV are already present in many areas of the world, serving as a reservoir to transmit these pathogens to naïve hosts. Despite the dangers that alphaviruses pose to human health there are currently no treatment options for any alphavirus infection besides supportive care.

Formation of 5' RNA cap structures on alphavirus RNA genomes via nsP1 guanylyltransferase / methyltransferase function is an essential component of alphavirus genome replication. 5' RNA caps direct translation of viral replication and structural proteins and protect genomic RNAs from degradation, and identification of chemical species that can specifically disrupt the RNA capping function of the alphavirus nsP1 protein can potentially provide novel interventions for these important human pathogens. To begin the process of identifying chemical inhibitors of alphavirus replication, we are developing and validating HTS and secondary screening assays that will be used to identify novel chemical inhibitors of the VEEV and CHIKV nsP1 enzymes, specifically inhibitors of nsP1 GTP binding and guanylyltransferase activity. We present progress towards purifying active VEEV and CHIKV nsP1 proteins and establishing a high-throughput fluorescence polarization assay to monitor GTP displacement from nsP1, and a fluorescent protein guanylation assays for use in validating HTS screens. This work will put us in an excellent position to begin screening chemical libraries for potent and specific nsP1 inhibitors.

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Antiviral Action of the Benzodiazols' Derivatives

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The creation of the new effective anti-influenza means as well as the instruments of their construction are the actual tasks of the contemporary medical science. The purpose of this study is to research antiviral activity of the benzodiazols' derivatives: 4-[(dimethylamino)methyl]-2-methyl-1H-1,3-benzodiazol-5-ol (C-60) and hydrochloride 2-methyl-4-(piperidin-1-ylmethyl)-1H-1,3-benzodiazol-5-ol (C-66). Methods of the compounds activity studies in vitro on the model tissue culture of chorio-allantoic covers of 10-12-days chicken embryos (CAC) and on the model of MDCK cell culture were used. Influenza viruses A/PR/8/34 (H1N1), A/Hong Kong/1/68 (H3N2) and avian H5N3 were solved in cultural media in the presence (experiment) and in the absence of compounds (control) to a concentration of 10,000(1x10⁴ log₁₀) TID₅₀. Control and experimental samples were incubated at 37oC for 24h. The number of infectious virus in the samples was determined by titration on fragments of CAC. Moreover antiviral activity was determined by infecting monolayers of MDCK cells with 10-fold dilutions of viruses A/PR/8/34(H1N1), A/Hong Kong/1/68(H3N2) in presence (experiment) or absence (control) of C-60 or C-66. Control and experimental samples were incubated at 37oC for 72h. Presence of virus was registrated in haemagglutination test. C-60 inhibited reproduction all of the strains viruses on the model tissue culture of CAC (A/Hong Kong/1/68 H3N2)-3,83, A/PR/8/34(H1N1)-4,0 and avian influenza (H5N3)-3,33 log₁₀ TID₅₀ as compared to control). This effect was detected also in cells culture MDCK (A/Hong Kong/1/68(H3N2)-3,33, A/PR/8/34(H1N1)-1,4 log₁₀ TID₅₀ as compared to control). C-66 showed higher antiviral activity on the model tissue culture of CAC (A/Hong Kong/1/68(H3N2) 5,17, A/PR/8/34(H1N1)-4,08 and avian influenza H5N3-3,83 log₁₀ TID₅₀ as compared to control). These data were not much higher than in cells culture MDCK (A/Hong Kong/1/68(H3N2)-3,33, A/PR/8/34(H1N1)-1,8 log₁₀ TID₅₀ as compared to control). So studied benzodiazols' derivatives show high level of anti-influenza activity in all used models. This research was supported by STCU Grant P407 -KCP T2-269

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Recombinant Neuraminidase Proteins for Identification of Potential Markers of Drug Resistance in Avian Influenza A(H7N9) Viruses

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The influenza A(H7N9) outbreak in China, which began in Spring 2013, emphasized the need for effective antiviral medications. All emergent A(H7N9) viruses contain the S31N substitution in the M2 protein, a common marker of resistance to the M2 blockers. Therefore, neuraminidase (NA) inhibitors (NAIs) are the only therapeutic option available for treatment of severe infections caused by A(H7N9). Two NAIs, oseltamivir and zanamivir, are approved for treatment of influenza infections in many countries, while peramivir and laninamivir are only licensed in a few. As therapeutic options are limited, monitoring for NA substitutions affecting susceptibility to one or more NAIs is essential.

To identify such substitutions, recombinant NAs (recNA) were generated using a baculovirus system optimized for expression of N9 NA. A series of mutations were introduced into the N9 NA, and resulting recNAs were tested in a NA inhibition assay with oseltamivir, zanamivir, peramivir, laninamivir, and A-315675. As predicted based on the similarities of the N9 and N2 NA crystal structures, substitutions known to affect NAIs susceptibility of N2 viruses were shared with the N9 enzyme. Specifically, R292K caused highly reduced inhibition by oseltamivir and peramivir and reduced inhibition by zanamivir and laninamivir in both N2 and N9 enzymes. RecNAs E119V and I222R had highly reduced inhibition by oseltamivir and those containing E119A/D/G, Q136K or E276D had highly reduced inhibition by zanamivir. In contrast, while H274Y in N2 does not affect oseltamivir inhibition, it did result in highly reduced inhibition in N9, similar to N1. Certain changes (e.g. I222R) affected inhibition by all NAIs. Notably, substitutions at residues 292, 119, and 222 have been detected in A(H7N9) viruses recovered from oseltamivir-treated patients. This report demonstrates the distinct drug susceptibility profile of A(H7N9) viruses. Furthermore, this study highlights the importance of antiviral testing of emergent influenza viruses; reinforcing the need of new anti-influenza drugs which target different aspects of the influenza virus genome and lifecycle.

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Characterization of the dynamics of human cytomegalovirus resistance to antiviral drugs by ultra-deep sequencing

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Prophylactic or preemptive treatments required to prevent human cytomegalovirus (CMV) infections in transplant recipients may fail when CMV drug resistance mutations (DRMs) are selected. Conversely to RNA viruses, a low level of genetic variability of DNA viruses, including CMV, has been reported, with very few circulating mixed populations. Thus, standard Sanger sequencing appears to be efficient to detect CMV resistance. However, a better knowledge of the dynamics of adaptation of CMV is needed to improve the detection of emerging drug resistance. This retrospective study aimed at describing CMV DRMs that developed over time using ultra-deep sequencing (UDS) in a case of CMV infection with transient response to different antivirals.

A kidney transplantation was performed in a 68 year-old CMV seronegative female with a CMV seropositive donor. She received different successive anti-CMV treatments: valganciclovir, foscarnet, and maribavir. More than 100 blood samples were collected over 2 years. When CMV load was above 745 IU/mL, full-length UL97 and partial UL54 CMV genes were sequenced by Sanger method and UDS after shotgun strategy using Roche 454 GS Junior sequencer.

A significant number of DRMs was detected over the study period. Minor variants were detected by UDS only, as their frequencies ranged from 2 to 20%: L405P, G598S (UL97); L501F (UL54). Other DRMs (>20%) were identified by UDS and Sanger method: H411Y, H520Q (UL97); K513E, G841A (UL54). Interestingly, the 2 latter mutations in UL54 conferred cross-resistance to ganciclovir/ cidofovir, and even foscarnet. Moreover, K513E was still detected despite the stop of antiviral treatments for a year, suggesting that the fitness of viral mutants was not impaired.

In conclusion, CMV revealed a complex dynamics of resistance under antiviral drug pressure, as described for quasi-species viruses. The emergence of successive DRMs constitutes a clinically challenging complication and contributes to difficult therapeutic management of patients.

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Co-circulation of Hepatitis A, B and C with Hepatitis E virus genotype 1a during major outbreak of 2014 in Nepal

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Nepal is one of the endemic countries for hepatitis E virus (HEV) outbreaks, which is associated with both epidemic and sporadic infections. To date, only two hepatitis E outbreaks have been studied. However, sporadic cases of HEV infection also take place through inter-epidemic period. The aim of this study was to detect the prevalence of HEV infection in patients with acute viral hepatitis (AVH). Blood samples and clinical information were collected between April and May 2014 from 211 patients who visited to Nobel Medical College and Teaching Hospital, Koshi Zonal Hospital and Biratnagar Hospital. Samples were tested for hepatitis B virus (HBV) surface antigen, anti-hepatitis C virus antibodies, anti-hepatitis A virus IgM, hepatitis E virus (HEV) antigen, anti-HEV antibodies (IgM and IgG) by ELISA and HEV RNA detection by real-time PCR. HEV was identified as the most common cause of AVH [36 (17.04%) of total 211 patients], followed by hepatitis A virus 8 (3.79%), HBV 7 (3.31%) and hepatitis C virus 1 (0.47%). Co-infections with more than one virus were found in 4 patients, with HAV-HEV the most common co-infection 3 (8.33%). Among 210 samples, 132 were detected for anti-HAV IgM, 117 samples for anti-HCV Ab and the overall positive rate of HEV RNA, anti-HEV IgM, anti-HBc Ab, anti-HAV IgM and anti-HCV Ab were 5.71%(12/210) 20.11% 36/179 6.06% 8/132 5.11%(7/137), 0.85% 1/117 respectively. To the best of our knowledge, this is the first documented epidemiological study of hepatitis outbreak in Biratnagar city, Nepal, indicating that this region is prevalent region for HEV infection. Sequence analysis of Hepatitis E virus shows all genotype belongs to HEV 1a. Phylogenetic analysis shows the virus originate from Karachi, South Pakistan, India and Myanmar.

54 In vitro Anti-Dengue Activity of Flavonoids

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BACKGROUND: Bioflavonoids are plant-derived polyphenolic compounds with many health benefits. Four types of bioflavonoids were selected and evaluated against dengue virus type -2 (DENV-2) in monkey kidney Vero cell line. Anti-dengue activities of these compounds were determined at different stages of DENV-2 infection and replication cycle. Foci Forming Unit Reduction assay (FFURA) and quantitative RT-PCR used to measure DENV replication.

RESULTS: It was shown that the IC₅₀ of quercetin is 35.7 µg mL⁻¹ when it was used after adsorption of DENV-2 to the cells. The IC₅₀ of quercetin decreased to 28.9 µg mL⁻¹ when the cells were treated continuously for 5 hours before virus infection and up to 4 days post infection. The SI values for quercetin were 7.07 and 8.74 µg mL⁻¹, respectively. Naringin only exhibited anti-adsorption effect against DENV-2 with IC₅₀= 168.2 µg mL⁻¹ and its related SI was 1.3. Daidzein showed a weak anti-dengue activity with IC₅₀=142.6 µg mL⁻¹ when the DENV-2 infected cells were treated after virus adsorption. The SI value for this compound was 1.03. Hesperetin did not show any antiviral activity against DENV-2.

CONCLUSION: The present study showed specific infection and replication processes targeted by various bioflavonoids. Among all tested bioflavonoids, quercetin demonstrated significant anti-DENV potentials and it should be considered for the development of new and more effective derivatives as anti-dengue therapeutics.

KEY WORDS: Antiviral, Dengue virus, Flavonoid, Quercetin, Naringin, Daidzein, Hesperetin

55 Impact of amino acid (aa) substitutions in influenza virus neuraminidase (NA) on resistance development against an orally bioavailable amidine derivative of oseltamivir

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NA inhibitors (NAI) are frontline therapeutics in the treatment of influenza but emerging resistant strains urge for the development of novel, effective drugs. Recently an orally bioavailable amidine derivative of oseltamivir was discovered which showed activity against an oseltamivir-resistant influenza A virus (Schade et al., 2014). Here we aimed at estimating the risk of resistance development for this oseltamivir analog. The influence of several mutations of influenza virus H1N1 A/WSN/33 NA was analyzed regarding drug susceptibility, enzyme kinetic parameters, and viral fitness. Oseltamivir, a guanidine analog thereof, and zanamivir were included for comparison. Viruses with potentially resistance-conferring single-aa substitutions (H274Y, Y155H, N294S, Q136L, I428M/Q), designed via a reverse genetics system, were analyzed for susceptibility against these NAI in chemiluminescence and fluorescence (FL) NA-inhibition assays. The aa substitutions resulted in specific levels of NAI resistance in both assays, but the FL assay appeared to be more sensitive to mutations. Guanidine and amidine analogs of oseltamivir were generally superior in their potencies measured by IC₅₀ values. They led to considerably reduced NAI resistance in case of H274Y. In contrast to the guanidine analog, the amidine did not improve the effect against N294S and Y155H. A predicted resistance conferring substitution I428Q decreased the effect of zanamivir and amidine oseltamivir by the same degree. According to km values determined in the FL assay, 428Q, 155H and 294S significantly affected substrate recognition of NA. These three A/WSN/33 mutants were also characterized by significantly reduced fitness in multistep viral replication cycle studies. Our results suggest a low risk of resistance development for the amidine analog of oseltamivir and enable further analysis of the underlying molecular mechanisms of action between NA and inhibitor to derive continuing strategies for developing resistance-breaking agents.

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5'-Monophosphates of 2'-deoxyuridine derivatives bearing extended C-5 alkynyl fragments: synthesis and biological properties

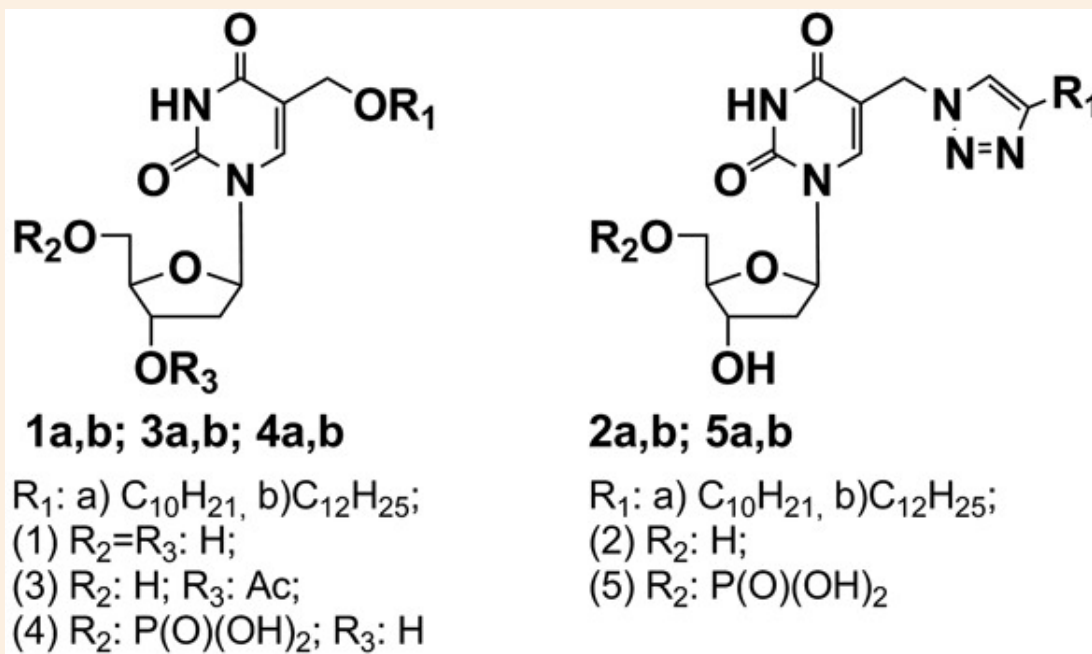
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Many 5-substituted derivatives of 2'-deoxyuridine inhibit the reproduction of herpes viruses (e.g., HSV and VZV)¹. Recently we synthesized two sets of 2'-deoxypyrimidine nucleosides bearing extended alkyloxymethyl *1a,b* or alkyltriazolidomethyl *2a,b* substituents at position -5 and demonstrated *in vitro* their effective bacteriostatic activity against *M. tuberculosis* strains². However, nucleosides with large hydrophobic fragments are insoluble in water that limits the biological investigations. The goal of this work was the synthesis and metabolism study of 5'-monophosphates of C-5 modified nucleosides.

Desired compounds *4a,b* were obtained involving a combination of protecting groups (*t*.butyldimethylsilyl and acetyl) to receive 5-modified-3'-acetyl-2'-deoxyuridines *3a,b* followed by phosphorylation with phosphoryl *tris*-triazole³ and deprotection. The 1,3-dipolar cycloaddition of 5-azidomethyl-2'-deoxypyrimidine 5'-monophosphate with olefins under the catalysis of Cu(I)⁴ resulted in compounds *5a,b*.

All compounds were not cytotoxic at concentrations up to 200 μM in *Vero* and up to 100 μM in *Jurkat* cells. The stability of the compounds to enzymatic hydrolysis was determined using fetal calf serum. All the compounds were stable under these conditions over a period 6 hours. The anti viral activity will be reported.



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57 Leveraging Your Strengths With Strategic Alliance Management

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More collaborative interactions are needed between academic institutions and biopharmaceutical companies to synergize available strengths and to enable the translation of laboratory discoveries into successful drug development programs. Academic institutions face significant hurdles in the drug discovery process, while pharmaceutical companies face thin pipelines and patent expirations. Bridging these sectors by forming alliances between academia and industry is a promising path forward but presents its own set of challenges. Due to the lack of priority setting, skills and tools, the success rate of alliances between these institutions is less than optimal (< 50% of alliances are defined as successful). BioLexUS, addresses this directly by providing the necessary tools, expertise and service to streamline alliance management and to extract more value and synergy by creating a win-win scenario for all the partners involved. We have addressed the frequent bottlenecks and provide solutions to address these impediments. Our findings and recommendations provide a fresh outlook on alliance management as an emerging science.

58 Bio-Activity Guided Fractionation and Characterization of Anti HSV-I molecule from *Nilgiranthus ciliatus* Nees (Acanthaceae)

Anjana Karunakaran Nair, Prasanth Francis, Manal Mohammed, Moolajogi Nanjan Chandrasekar, Ashish Wadhvani
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The widespread use of nucleoside based drugs had led to the emergence of resistance in Herpes simplex virus especially among immuno compromised patients. Therefore, new antiviral agents exhibiting different mechanisms of action are to be considered. *Nilgiranthus ciliatus* Nees (Acanthaceae) is widely used in Indian traditional system of medicine especially in Ayurveda for treating virus associated problems. The present study aimed to investigate the antiviral constituents of the plant by bioactivity guided fractionation. Materials and methods: Leaves stem and roots of *Nilgiranthus ciliatus* Nees (Acanthaceae) have been extracted using the solvents of non polar to polar and screened for cytotoxicity in Vero cell lines by MTT assay. Doses for antiviral study were calculated based on CTC₅₀ values and those screened against HSV-I of virus challenge doses 10TCID₅₀ and 100TCID₅₀ by Cytopathic Effect Inhibition (CPE) assay and MTT assay Results: Chloroform extract of roots of *Nilgiranthus ciliatus* Nees have shown maximum protection against HSV-I for 72 hrs at a concentration less than 50µg/ml and it has been subjected for further fractionation using column chromatography. NCR-I, NCR-II and NCR-III have been isolated and screened for its antiviral activity against HSV-I of virus challenge doses 10TCID₅₀ and 100TCID₅₀ by CPE assay. In this NCS-II has marked antiviral potential against both HSV-I and HSV-II. CPE assays were performed at various viral replication stages to find out the exact stage where the drug initiates its action. Structure of NCS-II has been elucidated using IR, NMR and MASS spectroscopy. Computer Aided docking studies have been carried out using the molecule (NCS-II) against possible viral targets. Conclusion: Given the pressing need for antiviral agents with new mechanism of action, the results in screening of biomolecules from the plant *Nilgiranthus ciliatus* Nees were promising and may provide a useful lead in the development of antiviral therapeutics. Further mechanistic studies are progressing in our laboratory.

59 Efficacy of isolated molecules from *Nilgiranthus ciliatus* Nees (Acanthaceae) against Herpes simplex virus type-I

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The widespread use of nucleoside based drugs had led to the emergence of resistance in Herpes simplex virus especially among immuno compromised patients. Therefore, new antiviral agents exhibiting different mechanisms of action are to be considered. *Nilgiranthus ciliatus* Nees (Acanthaceae) is widely used in Indian traditional system of medicine especially in Ayurveda for treating virus associated problems. The present study aimed to investigate the antiviral constituents of the plant by bioactivity guided fractionation. Materials and methods: Leaves stem and roots of *Nilgiranthus ciliatus* Nees (Acanthaceae) have been extracted using the solvents of non polar to polar and screened for cytotoxicity in Vero cell lines by MTT assay. Doses for antiviral study were calculated based on CTC₅₀ values and those screened against HSV-I of virus challenge doses 10TCID₅₀ and 100TCID₅₀ by Cytopathic Effect Inhibition (CPE) assay and MTT assay Results: Chloroform extract of roots of *Nilgiranthus ciliatus* Nees have shown maximum protection against HSV-I for 72 hrs at a concentration less than 50µg/ml and it has been subjected for further fractionation using column chromatography. NCR-I, NCR-II and NCR-III have been isolated and screened for its antiviral activity

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60 Regioselective synthesis of pyrazolo[3,4-d]pyrimidine based carbocyclic nucleosides as possible antiviral agents

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The infection of Hepatitis B virus (HBV) or Hepatitis C virus (HCV) is silent and remain asymptomatic for decades. Due to lower awareness, more than 80% HCV and over 60% patients with HBV are diagnosed at a stage when the disease is irreversible. This condition can lead to cirrhosis of the liver and finally to hepatocellular carcinoma. Several direct acting antivirals (DAAs) had been approved by US FDA for the treatment of these viral infections. However, due to resistance/side-effect/selectivity, there is still a need for exploration of newer analogs. Based on the previous encouraging results we have designed and synthesized a new carbocyclic nucleosides (I) based on 4-cyclopropylamino-1*H*-pyrazolo[3,4-*d*]pyrimidine as mimetics to act as antiviral agents through computer-aided approaches.

The pseudo-sugar moiety (carbocyclic) and the base were synthesized through long convergent synthesis of 6-8 steps each with 40-55% overall yields. The carbocyclic sugar and the base key intermediates were coupled by Mitsunobu reaction to get the N9 regiomers as major products. After column purification, deprotection was carried out to get the desired molecules. The biological evaluations of these compounds are under progress. (Acknowledgement: Authors thank DST and UGC for financial support)

61 Development of in silico drug resistance evaluation systems for anti-HIV drugs based on the target protein structures.

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Treatment of HIV infection has been improved day by day. However, drug resistance is still the big problems for the treatment nowadays. Therefore, it is important to take an appropriate drug selection for the treatment to drug resistant virus. Under such background, we reported *in silico* drug resistance evaluation methods to anti-influenza drugs recently. This method is combination of docking simulation and Boltzmann distribution, and evaluation was used for expectation value which is the sum of an existing probability of molecule-protein complex. Now we applied to anti-HIV drugs to extend the range of application of our method.

In this case, we evaluated drug resistance of atazanavir by using the complex structure with HIV protease. Thirty kinds of resistant mutations were selected from Stanford University HIV Drug Resistance Database. The mutation models were constructed from the known complex structure (PDBID: 2AQU) by homology modeling. MOE2013 was used for these calculations and MMFF94x forcefield was used. For docking simulation, the output was set 100,000 poses. London dG was used for scoring function and the other parameters were as default. Expected values was calculated by using appropriate docking poses.

Results of these calculations, the strong resistance strain showed low expected value. It was suggested that our evaluation was sufficient for this kind of resistance evaluation. Especially I84V related mutations, our method would be adequate prediction. On the other hand, it was not able to predict for V82A related mutations. It is currently under investigation for this kind of mutation.

Now we applied our method for atazanavir-resistant mutations, and the very strong resistant strains were calculated low expected value. It is shown that our method be sufficient evaluation. On the other hand, moderate resistant strains did not show exactly. It is necessary to further consider these kinds of mutations in the future.

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Identification of a broad-spectrum inhibitor of RNA viruses

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RNA viruses are so diverse, and are evolving so rapidly, that developing specific treatments against each one of them is impossible to achieve. A broad-spectrum inhibitor active against a large variety of RNA viruses would be ideal to fight both current and emerging RNA viruses. Although such a molecule is still a virologist's dream, a better understanding of cellular defense mechanisms and innate immune system suggests that possibilities exist. Several academic and industrial laboratories are now interested in discovering molecules that activate specific facets of cellular defense mechanisms, or perturb metabolic pathways, to hinder viral replication. To identify broad-spectrum antiviral molecules, we recently developed a high-throughput screening pipeline that combines *in vitro* infections by recombinant measles and chikungunya viruses, together with a cellular viability assay. This protocol was used to screen a commercial library of 10,000 molecules enriched for chemical diversity and select active compounds with a suitable profile. ChX-77 was identified as able to inhibit both measles and chikungunya viruses replication in HEK-293T cells (IC₅₀ = 100 nM). Cellular cytotoxicity was relatively limited, and the selectivity index, which corresponds to CC₅₀/IC₅₀ ratio, was estimated to 190. This compound was also active in other cell types (HEp-2 (human adenocarcinoma), MRC-5 (human lung fibroblast), RF/6A (endothelial cells of the retina)), and capable of blocking coxsackievirus replication in cell cultures. Finally, we explored the chemical space of ChX-77 structure by structure/activity relationship studies. Out of 140 tested analogs, 58 were found to be active against measles virus, with ChX-54 being twice more potent than ChX-77. This molecule has been selected for further investigations, and is currently evaluated for its capacity to block Ebola and Lassa virus replication in cell cultures.

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ACYCLOVIR 5'-HYDROGENPHOSPHONATE: ANTIHERPETIC PROPERTIES AND INTERACTION WITH OTHER NUCLEOSIDE DRUGS

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According to WHO, 90% of the planet population is infected by viruses of Herpesviridae family. The most prevalent of them is Herpes simplex virus type 1 (HSV-1), which establishes latent infection, but reactivates causing cutaneous or genital herpes, conjunctivitis, keratitis, encephalitis, eczema herpeticum, may be involved in multiple sclerosis pathogenesis [1] and may result in male infertility [2]. HSV often coinfects HIV-infected patients, moreover 6-10% bear virus strains resistant to available antiherpetic drugs. Most modern antiherpetic drugs are based on the use of modified nucleosides or their prodrugs [3]. Herein, we describe properties of acyclovir 5'-hydrogenphosphonate (HpACV) as antiherpetic agent. The compound inhibits the replication of ACV-resistant HSV strain in cell culture and moderately ACV-resistant clinical isolates. The compound protects HSV infected mice from death. The rate of development of HpACV-resistant strain was slower than that of ACV-resistant population. We compared the mutations in DNA polymerases and thymidine kinases of HSV strains resistant to HpACV and ACV and showed that the mutations are partly overlapped but HpACV resistant strains have individual set of mutations. The results mean that HpACV is not a prodrug of ACV. The study of antiherpetic activity of combinations of ACV or HpACV with other antiherpetic drugs will be presented. Acknowledgement. The work was supported by grants RFBR 13-04-40307-H and RAS Presidium (Molecular and Cellular Biology Program).

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64 Isolation and characterization of neutralizing single domain antibodies against junin virus

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Hemorrhagic viral fevers (HVF) are a group of diseases caused by different families of viruses. Some members of the Arenavirus family cause Hemorrhagic disease in humans, after the accidental contact with the infected natural host of these viruses. Argentine Hemorrhagic fever (AHF) is caused by the New World Arenavirus Junin (JUNV). Although for most of the HVFs only supportive treatment is available, transfusion of convalescent plasma is successfully used for AHF, reducing the mortality rate from 30% to 1%. Nevertheless, this treatment has limitations such as troubles in maintaining adequate stocks of immune plasma and its lack of efficacy in severely ill patients.

In this work we explored the development of a novel and improved therapeutic intervention against AHF, based on the generation and use of single domain antibodies (Nanobodies® or VHH). This technology combines unparalleled antigen-targeting specificities with ease of expression, purification and re-engineering opportunities.

The generation of the nanobodies directed against JUNV was performed by immunizing an alpaca with purified UV-inactivated Candid#1 JUNV. The JUNV-binding capacity of the VHHs was evaluated by ELISA. Next we determined the biological activity of these binders in a virus neutralization assay against JUNV Candid#1 strain. Three out of 70 VHH candidates were able to neutralize successfully Candid#1 and one related attenuated strain, but not the pathogenic strains XJ and CbaIV4454. When the target of the neutralizing nanobodies was studied, we observed that the viral nucleoprotein was recognized by these VHHs. This is surprising because conventional antibodies directed against JUNV nucleoprotein are not known to be neutralizing.

Although Candid#1 neutralizing VHHs did not show activity against the pathogenic strains, these findings constitute the first demonstration that nanobody technology could be used to neutralize JUNV replication. Also these results indicate that, for the first time, the Arenaviral nucleoprotein is a probable antiviral target for neutralizing nanobodies.

65 Potent Broad Spectrum Anti-DNA Viral Activity for HPMPA and HPMPA Tyrosinamide Prodrugs

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The broad spectrum antiviral activity of acyclic nucleoside phosphonates (ANPs) makes this prominent class of therapeutics useful agents in the treatment of many DNA virus infections. The prototypic molecules cidofovir ((S)-HPMPC, Vistide®) and ((S)-HPMPA) exhibit antiviral activity against a wide variety of DNA viruses, including the herpesviruses, poxviruses, polyomaviruses and papillomaviruses. A major hindrance in meeting their therapeutic potential is the presence of an ionizable phosphonic acid group, which results in poor cell membrane permeability and low oral bioavailability at physiological pH. We previously reported the synthesis and preliminary studies of a series of lipophilic tyrosinamide prodrugs of (S)-HPMPA and (S)-HPMPC. Here we present structure activity relationships of alkyl group size in a set of 8 viruses including representatives of each of the four virus families described above. In all viruses, increased length of the alkyl moiety correlated well with increased potency with maximal activity observed with alkyl chains of at least 14 carbon atoms in length. In the HPMPA series, up to 30-fold improvements were observed in the therapeutic indices for some viruses and were virus specific. The most marked improvements in antiviral activity were observed against vaccinia virus, varicella-zoster virus and human papillomavirus and only modest changes occurred against herpes simplex virus and adenovirus. This strategy has the potential to greatly improve the activity ANP and suggests viral infections that might be best targeted with this approach.

ACKNOWLEDGEMENTS: This work was supported by NIH Grant R43 AI100401 and Public Health Service Contract HHSN27200005, NIAID, NIH.

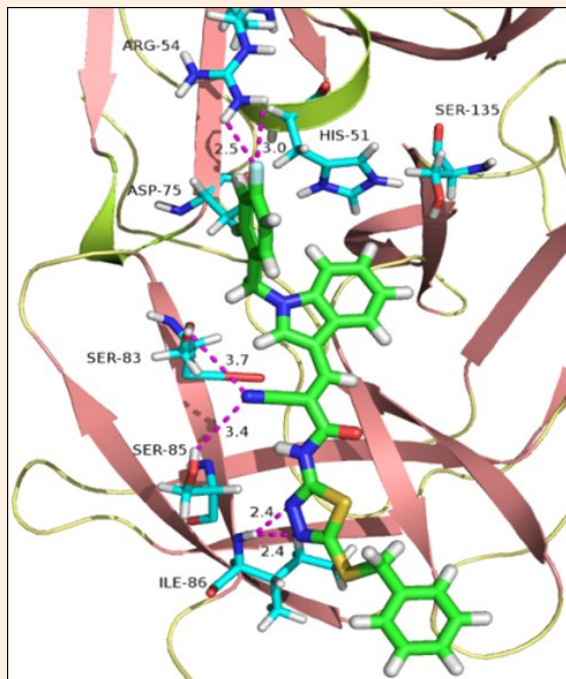
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Structure-based Design of Novel Thiadiazoloacrylamides as Flavivirus Protease Inhibitors

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The NS2B/NS3 protease is a promising but challenging target for the development of antivirals against flavivirus infection. Here we present our efforts to develop novel thiadiazoloacrylamide inhibitors targeting this protease. Initially, a DENV NS2B/NS3 protease inhibitor with thiadiazolopyrimidinone scaffold was identified by screening a commercial library. Optimization of this hit led to the finding that the thiadiazoloacrylamide core displays both better activity and superior pharmacokinetic properties. Further modifications of the core skeleton afforded 14 compounds with good activity against DENV-2 NS2B-NS3 protease, with the best of them displaying IC₅₀ = 2.24 ± 0.32 µM. Docking studies gave insights into the binding modes and showed that the nitrile group in the linker part is essential for obtaining good inhibitory activity. Determination of the inhibitory activities of the thiadiazoloacrylamide derivatives against WNV NS2B-NS3 protease is ongoing. In addition, two of the most active compounds were co-crystallized with both WNV NS2B-NS3 protease and full-length DENV-4 NS2B-NS3 protease/helicase. We hope to present crystal structures of these complexes, which will provide a more solid basis and detailed guidance for further “hit to lead” processing.



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New 4(1H)-Quinolinone Derivatives to Defeat Chikungunya Virus.

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Chikungunya is one of the most important re-emerging human arboviral infections of global significance. Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) and is transmitted to human primarily by *Aedes aegypti* mosquitoes, the infamous yellow fever propagator.^{1,2} The symptoms generally start 4–7 days after the bite. Acute infection lasts for 1–10 days and is characterized by abrupt onset of fever, headache, fatigue, nausea, vomiting, rash, myalgia, and severe arthralgia. Despite the gravity of its infectious potency, there is currently no antiviral treatment or vaccine against CHIKV infection. Previous studies have reported anti-CHIKV activities in vitro for some compounds.^{3–5} Among these, only chloroquine has been tested in vivo but with poor results. For these reasons, there is an urgent need for the discovery of antivirals active against CHIKV infection. For many years we focused our research in the field of anti-infective agents, in particular to find compounds active against HIV,⁶ HCV,⁷ and influenza A.⁸ Recently, we undertook a screening for the identification of novel anti-HCV agents and widened the test with the aim to discover compounds able to block the CHIKV replication. The preliminary data will be shown and discussed.

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Testing anti-HIV activity of antiretroviral agents in vitro using infection of CEM-GFP cells with an HIV-1 NL4-3 recombinant strain and flow cytometry

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Here we describe an assay, specifically optimized in our laboratory to evaluate the anti-HIV activity of antiretrovirals. Zidovudine (AZT), abacavir (ABC), 2',3'-dideoxyinosine (DDI), lamivudine (3TC), nevirapine (NVP), efavirenz (EFV) and delavirdina (DLV), were assayed as licensed drug for their anti-HIV activity. Stocks of HIV-1 NL4-3 virus, were prepared by transfection of HEK293T cells with purified plasmid DNA. Viral particles were purified, concentrated by ultracentrifugation and quantified by p24 antigen-capture assay. For infection, CEM-GFP cells (1×10^5 cell) were resuspended in 50 μ l of RPMI 1640 and unexposed or exposed to several concentrations of drugs (from 0.1 to 100 nM) for 2 hours at 37°C before purified HIV-1 NL4-3 was added to each sample by spinoculation at 400 g for 1 h at r.t. Then, the adsorption was prolonged for 3 h at 37°C. Then cells were washed to remove virus excess before the same concentrations of drugs was replenished. After 72 h incubation, cells were harvested, centrifuged and fixed in 2% formaldehyde/PBS. HIV-induced GFP expression in infected CEM-GFP cells was then assessed by flow cytometry analysis. For comparison, p24 production in supernatants was evaluated by ELISA kit. (HIV-1 p24 ELISA). On the basis of IC₅₀ values, the anti-HIV activity, as assayed by the method we set up, where EFV > 3TC > AZT > NVP > DDI > ABC > DLV, used at the nanomolar range. The comparison between the IC₅₀ values calculated through flow cytometry and p24 production revealed overlapping results, showing that infection of CEM-GFP with HIV NL4-3, according to the protocol we set up, can be a suitable model in the perspective to perform quantitative, rapid and low-expensive screening tests to evaluate the in vitro effect of new antiretrovirals towards HIV cell-free infection.

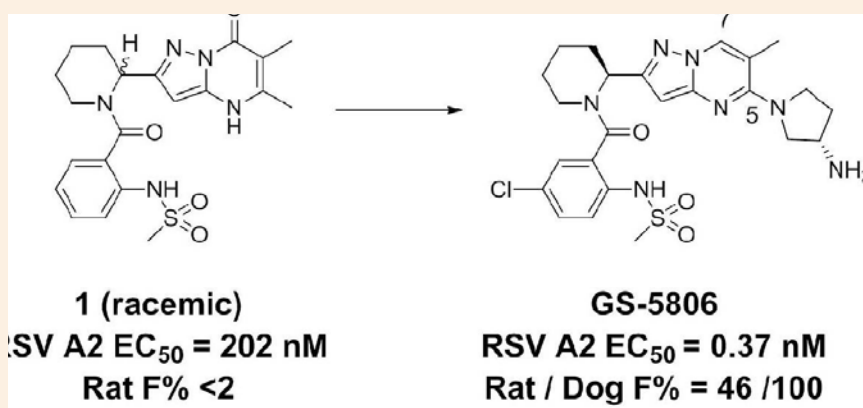
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Oral Respiratory Syncytial Virus (RSV) Fusion Inhibitor GS-5806: Potency and Oral Pharmacokinetics Optimization

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RSV is a common seasonal virus that most severely affects infants, immunosuppressed patients, and the elderly with cardiovascular disease. It is the leading cause of infant hospitalizations and there are no effective treatment options. GS-5806 is a novel, once daily, oral RSV fusion inhibitor that has been discovered following a lead optimization campaign on a screening hit 1. Oral absorption properties were improved by switching to the pyrazolo[1,5-a]pyrimidine heterocycle. Modifications on the pyrimidine ring resulted in analogs with improved hepatic stability and human plasma adjusted (pa) potency. Introduction of basic amines afforded favorable lung/plasma exposure ratios in Sprague-Dawley (SD) rats supporting the candidate selection of the aminopyrrolidine analog GS-5806. Potency and pharmacokinetic properties of GS-5806 include a paEC₅₀ = 8.1 nM (0.37 nM [EC₅₀] x 22 [plasma shift]), oral bioavailability ranging from 46% (rat) to 100% (dog), and a lung/plasma AUC_{0-12h} ratio of 26-fold in SD rats. GS-5806 exhibited potent in vitro activity against 75 RSV A and B clinical isolates (mean EC₅₀ = 0.43 nM). In a RSV challenge study GS-5806 demonstrated a significant antiviral and symptomatic treatment effect at trough plasma exposures exceeding paEC₉₅. GS-5806 is currently being evaluated in phase 2 studies of natural RSV infection.



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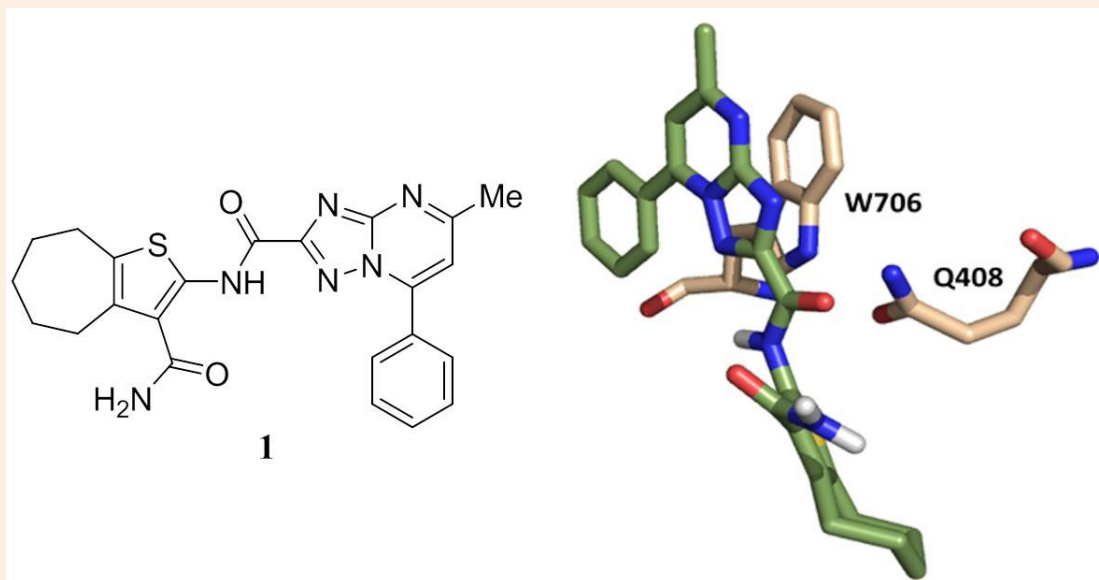
Identification of a Hybrid Small Molecule that Potently Disrupts the Polymerase PA-PB1 Subunits Interaction and Shows Broad Anti-Flu Activity

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The limited therapeutic options against influenza (Flu) infection along with the drug resistance issue make imperative the search for next-generation agents. Although the inhibition of viral polymerases is a common approach in the identification of antiviral drugs, the development of compounds able to interfere with the correct assembly of viral polymerase complexes is an innovative strategy. In this context, we have been recently interested in the inhibition of Flu polymerase PA-PB1 subunits interaction by small molecules.^{1,2} Starting from a dihydrotriazolopyrimidine derivative, previously identified thanks to a SBDD, and through a hit-to-lead optimization, novel potent inhibitors have been recently discovered. The structure modifications performed around the bicyclic core have led to compounds endowed with both the ability to disrupt PA-PB1 subunits interaction and broad anti-Flu activity with no cytotoxicity. The most interesting results were obtained with hybrid molecules designed by merging some peculiar structural features known to impart PA-PB1 interaction inhibition, with compound 1 that emerged as the most potent in the scenario of the PA-PB1 small molecule inhibitors developed so far.

The design, synthesis and anti-Flu profile of the triazolopyrimidine derivatives will be the object of the presentation.



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Anti-Influenza Activity of a New Coordination Compounds

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The creation of the needed chemical-therapeutic reserve which could provide the prophylactics and treatment in the case of the significant increase of influenza infected persons is the actual task of the contemporary medical science. Now intensively studied properties of metal complexes for detection of new biological properties to create a new anti-infection drugs.

New coordination compounds of cobalt bis(citrato)germanate (Ge) and cobalt bis(citrato)stannate (Sn) have a low toxic that gives the prospect for further research and implementation in medical practice.

Methods of the compounds activity studies in vitro on the model tissue culture of chorio-allantoic covers of 10-12-days chicken embryos (CAC) and on the model of MDCK cell culture were used. Influenza viruses A/PR/8/34 (H1N1), A/Hong Kong/1/68 (H3N2) and avian influenza H5N3 were solved in cultural media in the presence (experiment) and in the absence of compounds (control) to a concentration of 10,000(1x10⁴ log₁₀) TID₅₀. Control and experimental samples were incubated at 37°C for 24h. The number of infectious virus in the samples was determined by titration on fragments of CAC. Also antiviral activity was determined by infecting monolayers of MDCK cells with 10-fold dilutions of viruses A/PR/8/34(H1N1), A/Hong Kong/1/68(H3N2) in presence (experiment) or absence (control) of Ge and Sn. Control and experimental samples were incubated at 37°C for 72h. Presence of virus was registrated in haemagglutination test. Antiviral activity of Ge was significantly higher to Sn against the strain of influenza virus A/Hong Kong/1/68(H3N2). Ge inhibited the reproduction of the virus to 5,25 IgTID₅₀, Sn – 4.33 IgTID₅₀. Ge and Sn inhibit reproduction of A/PR/8/34(H1N1) at lower concentrations. Sn inhibited the reproduction of H5N3 bird virus is much stronger than Ge. Sn showed more pronounced antiviral activity against virus A/Hong Kong/1/68(H3N2) and A/PR/8/34(H1N1) in cell culture MDCK.

So, new coordination compounds have antiviral activity against human influenza virus strains A/Hong Kong/1/68 (H3N2) and A/PR/8/34 (H1N1), as well as the avian influenza virus H5N3

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A new rapid and accurate method for screening plants extracts for antiviral activity

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A large number of human diseases are caused by viruses yet the current antiviral therapies are restricted to a small number of viral diseases. Consequently, there is an urgent medical need for new and safe antiviral drugs. Plants and fungus are a good natural source for antiviral agents. We recently developed a fast and reliable screening method to identify new potential antiviral drugs derived from plant extracts [S. Matza-Porges et al, Journal of Virological Methods (2014), 208:138-143]. The method consist of two steps: the first step detects the extract's toxicity to the cells and the second step identifies inhibition of viral replication directly via reporter gene expression. In the present study we further developed the two-step method to detect inhibition of viruses independently from a reporter gene viral construct. In this improved method, the second step identifies inhibition of viral replication via immunocytological assay in which viral viability is tested by immuno-testing for the expression of the virals glycoprotein on the surface of the infected cell. The combination of the two independent virus propagation detection methods is suitable for screening antiviral substances. Utilizing these methods, we screened 92 plant extracts of which 12 were tested using the immunocytological assay. The results obtained were in correlation with the results of the recombinant HSV-1 expressing GFP. Overall, six extracts showed potential to exert specific HSV-1 growth inhibition activity. Both the leaves and stems of *Sedum sediforme*, and the leaves and flowers of *Fumana thymifolia* showed anti-HSV-1 activity. This method enables rapid and accurate detection of potential antiviral substances against HSV-I and other viruses.

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Selective inhibitors of PI4K III as broad spectrum antiviral agents

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INTRODUCTION: Phosphatidylinositol 4-kinases (PI4Ks) are important cellular enzymes that catalyse the phosphorylation of phosphatidylinositol, a constituent of cell membranes, into phosphatidylinositol 4-phosphate (PI4P), a key signalling molecule associated with membrane budding and vesicular transport. There are four distinct types of human PI4Ks divided into two classes (class II and class III). A number of (+)ssRNA viruses hijack the members of PI4K class III in order to rebuild membranes inside of host cells and establish functional replication machinery. In particular, PI4K III is utilized by numerous members of the *Picornaviridae*, *Coronaviridae* and *Flaviviridae* families.

METHODS: We synthesized a series of more than 65 compounds as potential inhibitors of PI4K III and evaluated their inhibitory activity against PI4K III and two other members of the PI4K family (PI4K III, PI4K II). We screened this library of compounds against selected (+)ssRNA viruses (two serotypes of HCV, Coxsackie virus B3 and Human Rhinovirus). Several inhibitors were also subjected to co-crystallization with the title enzyme in order to obtain structural information on this complex.

RESULTS: Among the synthesized compounds we identified a number of highly potent inhibitors of PI4K III with the most interesting compound being MI14 ($IC_{50} = 54 \pm 15$ nM). MI14 exerted unprecedented selectivity within the PI4K family with the IC_{50} of both tested PI4Ks greater than 100 μ M. In addition, MI14 displayed antiviral activity against members of both the *Picornaviridae* and the *Filoviridae* families with activities being in the nanomolar level for CVB3 and HCV 1b and in low micromolar level for HRV, and HCV 2a.

CONCLUSION: We identified an extremely selective inhibitor of PI4K III, which significantly inhibited replication of several important viral pathogens. Based on crystallographic studies and molecular modelling we were able to explain the mode of action of this compound.

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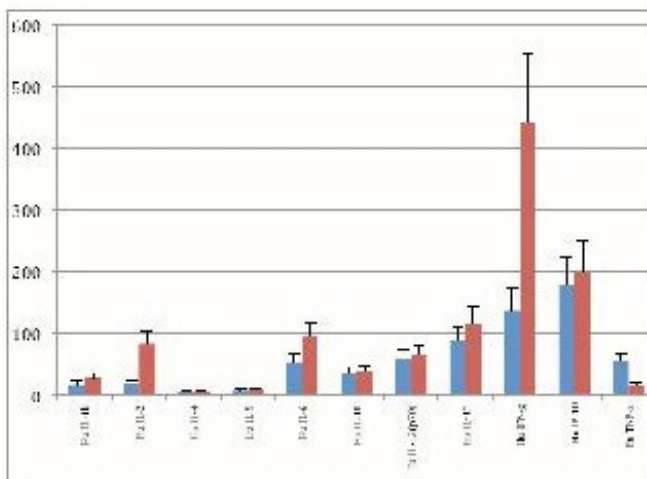
Poly-functional immune response in HIV infection: a correlation between immunological and clinical status

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Aim: We analysed patient immunological status and serum cytokine profile to establish immune-activation state and afterwards the obtained data were correlated to viral activity and ARV therapy. **Materials and Methods:** 50 HIV-1 mono-infected patients (pt) were recruited: 5 naïve, 5 virologic controllers, 10 virologic non controllers (ARV drug-untreated pt with HIV viremia above 10,000 copies/ml), 20 virologic responders (ARV drug-treated pt with undetectable HIV viremia) and 10 virologic non responders patients (ARV drug-treated pt with HIV viremia ≥ 400 copies/ml). IFN-g production and plasmatic cytokine pattern levels (IL-1, IL-2, IL-6, TNF-, IL-4, IL-10, IL-13, IP-10, IL-8, MIP-1, MIP-1, MCP-1) were assayed on admission and 12 months follow-up. **Results:** Our data showed that patients with control of HIV-1 viremia independently of ARV showed higher levels of polyfunctional IFN-g/IL-2 producers gag-specific CD4 and CD8 T cells, compared to subjects with progression of HIV infection that showed a predominance of single IFN- producers gag-specific T-cells. Figure 1 displays that non responder patients showed higher levels of inflammatory cytokines as compared to responders. **Conclusions:** Our data suggest that the presence of poly-functional immune response is a strong correlation of HIV-control; the analysis of T-cell immune response together to cytokine profile focus not only on HIV immunopathology but could support the clinicians to apply different approach strategies to manage HIV-1 infection.

Fig. 1 Average cytokines levels in “responders” (in blue) and “non responders” (in red), during the period of study



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Antiviral activity of the medicinal serum containing metabolites of baicalein as anti-dengue virus agent

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Dengue virus is the most common mosquito-borne human disease and it is leading to dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Till now there is no effective antiviral drug or vaccine against dengue available. Therefore, finding the efficient antiviral against this virus is very crucial.

Flavonoids are polyphenolic plant with various biological properties. Based on previous studies, it was demonstrated that some flavonoids including baicalein exert antiviral activity against DENV *in vitro*. However, it was shown that baicalein after administration in animal models is converted to different metabolites such as baicalin. Therefore, it is necessary to evaluate the antiviral properties of this metabolite beside its bioavailability in animal model through different routes of administration. Hence, in the present study, first we have evaluated the *in vitro* anti-DENV activity of baicalin followed by evaluation of the different routes of administration for baicalein including oral and intraperitoneal administration in SD rat as animal model to determine the best route of administration for baicalein. Our findings showed that baicalin as the main metabolite of baicalein exhibits significant *in vitro* anti-DENV activity at $IC_{50}=13.5$

± 0.08 mg/ml. It was also demonstrated that oral administration is the best route of administration for baicalein. We have found that medicinal sera with 1/2 concentration can neutralize DENV replication efficiently with 76% inhibition in cell culture.

In conclusion, further investigations on baicalein and baicalin as anti-DENV candidates is warranted.

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Synthesis and in-vitro antimicrobial activity of novel 2-nitro benzoic acid derivatives

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A diversity of biological activities and pharmaceutical uses have been attributed to benzoic acid derivatives, such as antibacterial, antifungal (1, 2). A series of 2-nitro benzoic acid derivatives were synthesized and their structure confirmed by FT-IR, ¹HNMR, ¹³ CNMR and elemental analysis.

In vitro antimicrobial activity of compounds was determined against *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumonia* ATCC 700603, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 43071, as (Gram-negative) bacteria and *Enterococcus faecalis* ATCC 29212, *Staphylococcus* ATCC 25952 as (Gram-Positive) bacteria was done by use of paper disc diffusion method on Mueller Hinton agar (Merck). Chloramphenicol and Ciprofloxacin were standard reference antibiotics. The zone of inhibition against bacteria was measured after 24 hours at 37 C. These compounds shows high to moderate antibacterial activity.

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Purposeful Search and Structural Drug Design of New Anti-Influenza Agents using HiT QSAR

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The development of selective anti-influenza agents by means of QSAR modeling of antiviral activity of the diverse set of chemical compounds has been continued.

The QSAR modeling was carried out using Hierarchic QSAR Technology (HiT QSAR) based on Simplex representation of molecular structure and random forest statistical method. Different statistical models has been developed and refined during QSAR investigations:

- the toxicity on *Colpoda steinii* culture (85 compounds);
- inhibition of reproduction of the influenza virus H5N3 (54 compounds).
- inhibition of reproduction of the influenza virus H1N1 (A/PR/8/34) on CAM tissue culture (compound 41);
- inhibition of reproduction of the influenza virus H1N1 (A/PR/8/34) in cell culture MDCK (27 compounds);
- study of chemotherapeutic index on influenza virus strain H3N2 (A / Hong Kong/1/68) in MDCK (37 compounds);
- study of chemotherapeutic index on influenza virus strain H1N1 (A/PR/8/34) in MDCK (37 compounds).

Models are adequate, have good predictive ability and wide domain applicability. As a result of QSAR studies two compounds are recommended for further researches: 4-[(dimethylamino)methyl]-2-methyl-1H-1,3-benzodiazol-5-ol and hydrochloride 2-methyl-4-(piperidin-1-ylmethyl)-1H-1,3-benzodiazol-5-ol has been recommended for further experimental testing. The results of experimental testing shows high influenza activity in 10^{-3} M against different strains of the virus in CAM tissue culture compared to the reference drug Tamiflu. The results of docking shows that all active compounds derived from QSAR studies have ability of binding with receptor of hemagglutinin, all of these compounds demonstrate the ability to dock to the same site as rimantadin. This demonstrates necessity of further researches of recommended compounds individually and in combinations.

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In vitro selection and characterization of influenza A virus variants resistant to a novel polymerase inhibitor of PA/PB1 interaction

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The major limitation of the approved anti-influenza drugs is the rapid emergence of drug-resistant viral strains. Recent studies from our group have led to the identification of small molecules endowed with anti-influenza activity, including the Hit 1 (Muratore *et al.*, Proc. Natl. Acad. Sci. USA 2012, 109:6247-52). These inhibitors were able to interfere with the interaction between PB1 and PA subunits both *in vitro* and in cells, as well as transcription by the RNA polymerases of influenza viruses. To further assess their therapeutic potential, the propensity of our PA-PB1 inhibitors to develop drug resistance has been investigated. To this end, a strain of influenza A virus (A/PR/8/34) has been sequentially passaged in confluent MDCK cells in the presence of increasing concentrations of compound 1. Parallel passaging experiments were conducted with oseltamivir carboxylate, the active form of the most widely prescribed agent for the treatment of influenza virus infections. After each passage, the viral progeny was titrated and the susceptibilities to both drugs were determined by plaque reduction assays (PRAs). To confirm the onset of the drug resistance, PRA was also conducted using A/PR/8/34 wt virus as a control. Passage experiments with oseltamivir identified a variant at passage 4 that was 5000-fold less susceptible to the compound. By comparison, virus variants recovered from passaging with Hit 1 showed a significant reduction of susceptibility (5-fold) to the PA/PB1 inhibitor only after the passage 12. These results suggest that PA/PB1 inhibitors might exhibit considerably lower propensity to develop drug resistance than other anti-influenza compounds. Then, the resistant viruses were picked from the plaques and subjected to three rounds of plaque purification in the presence of the same concentration of drugs. Sequencing of the viral population in order to identify the nucleotide mutations associated with drug resistance is ongoing.

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Antiviral activity of natural phytochemicals against noroviruses

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Human noroviruses (HuNoVs) are known as a major cause of acute gastroenteritis epidemics worldwide. Since HuNoVs cannot be cultured, murine norovirus-1 (MNV-1) has been widely accepted to be the best surrogate system of HuNoVs. In this study, the effect of natural substances including plants extracts, essential oils and essential oil-derived single components were investigated on the infectivity of murine norovirus-1 (MNV-1) using plaque reduction assay. MNV-1 (10^5 pfu/mL) was incubated with natural substances (1 mg/mL or 0.2% v/v), and incubated for 72 h at 4 °C. The infectivity of the recovered viruses after treatment was evaluated by plaque formation. Among 100 tested natural substances, the extracts from *Gleditsia spina*, *Aloe spp.*, *Zedoariae Rhizoma*, *Indera obtusiloba Blume*, *Eriobotriae Folium*, and *Lemongrass* essential oil have been identified to be effective in inactivating the infectivity of MNV-1. Antiviral activity of these extracts was maintained at the room temperature (25 °C) and exerted with as short as 30 min of incubation. IC_{50} of these extracts were estimated to be 0.306, 1.381, 3.315, 4.044, 4.058 mg/mL, and 0.15 %, respectively. Although further studies should be warranted to identify the responsible phytochemicals and elucidate the mechanism of action of these bioactive substances, our results suggest that bioactive phytochemicals may help to directly inactivate norovirus particles.

80 **Activity of a Triple Antiviral Combination Administered by Consecutive Alternative Treatment Scheme Against Coxsackievirus B1 Infection in Mice**

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In previous study we have reported the marked antiviral effect in newborn mice infected s.c. with 20 LD₅₀ of Coxsackie virus B1 (CVB1) of a treatment course by a triple combination of enterovirus replication inhibitors pleconaril/guanidine/ oxoglacine (PGO) following consecutive alternative administration (CAA) scheme. When the infectious virus content in the mouse brain isolates infected with CVB1 and treated with combination PGO applied consecutively was determined, a distinct reduction of the virus titer was found after day 4 post virus inoculation and attaining maximal decrease at days 11th – 13th. At the same time the monotherapy with pleconaril 25 mg/kg daily evoked a moderate virus titer decrease at days 4-6th post infection, followed by a marked decrease of the antiviral effect, what could be classified as appearance of drug-resistance. This phenomenon was confirmed through the measuring the values of the minimal inhibitory concentration (MIC₅₀) by using the plaque inhibition test in HEP-2 cell cultures. A marked increase of MIC₅₀ values was registered in brain samples taken since day 7th, attaining its maximum at day 13th. The CAA course with the PGO triple combination prevent completely the development of drug resistance till the end of the observation period (13th day post infection). The application of the triple combination (PGO) every day brought to a quicker development of resistance.

The data obtained are new proves in favor of the CAA course perceptiveness for reaching the clinically effective chemotherapy of enterovirus infections.

81 **Preliminary Evaluation of Anti-Poliovirus Effect of two Lactic Acid Bacteria in an in vitro assay system**

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Orally ingested probiotic bacteria have been reported to offer beneficial effect against enteroviruses, some studies have reported poliovirus response to standardized probiotics challenge but no report of such studies have been documented in Nigeria. Nigeria remains one of the four countries of the world where poliovirus transmission has never been interrupted. The aim of this study to investigate the effect of lactic acid bacteria; *Enterococcus durans* and *Weissella confusa* isolated from local food sources on poliovirus replication in tissue culture system. Broth culture containing 108 CFU/mL of the two bacteria were screened against the three poliovirus serotype (PV1, PV2 and PV3). Bacteria strains were introduced into a 24 hour monolayer culture of Rhabdomyosarcoma (RD) cells both pre and post inoculation with poliovirus. After incubation for 72h, cell viability was determined using the MTT assay. Result obtained indicated different degrees of poliovirus inhibition by both bacteria strains; specifically strains that were introduced pre poliovirus inoculation exhibited a higher degree of antiviral activity of greater than 80% in the three PV serotype while bacteria that were introduced post inoculation showed a lesser degree of antiviral activity with less than 45% inhibition. These preliminary results indicate potential beneficial effects of lactic acid bacteria in combating poliovirus and other enterovirus infections.

82 **Selective inhibition of HIV-1 replication in latently infected cells by the CDK9 inhibitor FIT-039**

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HIV-1 Tat recruits CDK9/Cyclin T1, a subunit of positive transcription elongation factor b (P-TEFb), and promotes HIV-1 transcription. The cellular transcriptional factors SP1 and NF- B, which activate basal HIV-1 transcription by binding to HIV-1 long terminal repeat, also recruits CDK9/Cyclin T1 independently of Tat. Therefore, CDK9/Cyclin T1 is an attractive target for inhibition of HIV-1 replication by small-molecule compounds. In this study, we investigated the anti-HIV-1 activity of the novel CDK9 inhibitor, FIT-039. The compound was shown to inhibit replication of a broad spectrum of DNA viruses, including HSV-1, HSV-2, human adenovirus and cytomegalovirus [1]. FIT-039 suppressed HIV-1 production induced by TNF- from the HIV-1 latently infected cells OM-10.1, U1, and ACH-2 in a dose-dependent manner. Its EC₅₀ for HIV-1 replication were 1.4 ± 0.7, 1.8 ± 0.3, and 2.1 ± 0.6 μM in OM-10.1, U1, and ACH-2 respectively. In contrast, the CC₅₀ were > 20 μM for all cell lines. FIT-039 also showed inhibition of HIV-1 gene expression induced by TNF- in OM-10.1 cells in a dose-dependent fashion. However, FIT-039 did not show significant inhibition of HIV-1 replication in acutely infected peripheral blood mononuclear cells. Since the activation of basal transcription of HIV-1

by cellular transcriptional factors plays a more important role in HIV-1 gene expression in latently infected cells than in acutely infected cells, we investigated the effect of FIT-039 on NF- κ B activation in TNF- α -stimulated OM-10.1 cells and found reduced NF- κ B translocation into the nucleus and increased I κ B expression in the cytoplasm. Thus, FIT-039 presumably targets NF- κ B-dependent CDK9 activation and inhibits HIV-1 replication in chronically infected cells. 1. Yamamoto, M., et al. CDK9 inhibitor FIT-039 prevents replication of multiple DNA viruses. *J. Clin. Invest.* 124, 3479-3488, 2014.

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Formation of Efavirenz cocrystals from Stoichiometric Solutions of Incongruently Saturating Systems by Spray Drying Technology.

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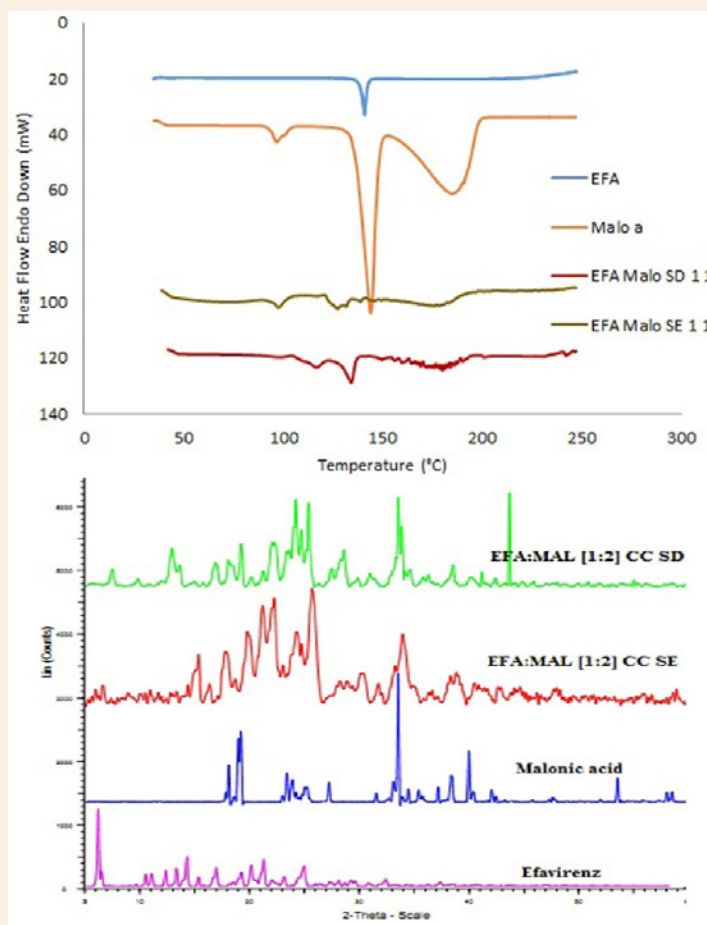
BACKGROUND: Poor biopharmaceutical performance of BCS class II drugs is a major hurdle in design and development of pharmaceutical formulations. Up to date, cocrystals have been an emerging formulation approach aiming to improve the solubility, dissolution, bioavailability and stability.

METHOD: In the present investigation, we explored spray drying as a potential novel technique for cocrystallization of Efavirenz (EFA). Methanolic solution consisting of EFA and coformer like Malonic acid in equimolar proportion was sprayed at temperature of 50-51 °C, feed rate of 2ml/min and vacuum was maintained at 50 m³/h. The obtained cocrystalline powder was stored in desiccator. Prototype cocrystals of the same system were made by solvent evaporation method. DSC and PXRD studies were performed to characterize the physical form of EFA in cocrystalline product. Hot stage microscopy (HSM) was conducted to determine the thermal transitions of drug upon heating. The product was filled into capsules and dissolution studies were performed along with pure EFA and physical mixtures as per USP monograph.

RESULTS: The DSC studies indicated that both the carrier and drug maintained in the crystalline state after the spray drying process, which was further confirmed by PXRD. (HSM) was conducted to visually determine the thermal transitions and extent of drug melting in cofomers. DSC results are in good agreement with HSM findings. The cocrystals of EFA with Malonic acid in 1:2 equimolar proportion showed a 3.6-fold higher release rate compared to the pure drug.

CONCLUSION: In the present investigation, spray-drying technique was successfully utilized for the generation of cocrystals EFA with Malonic acid.

KEYWORDS: Efavirenz, cocrystals, spray drying.



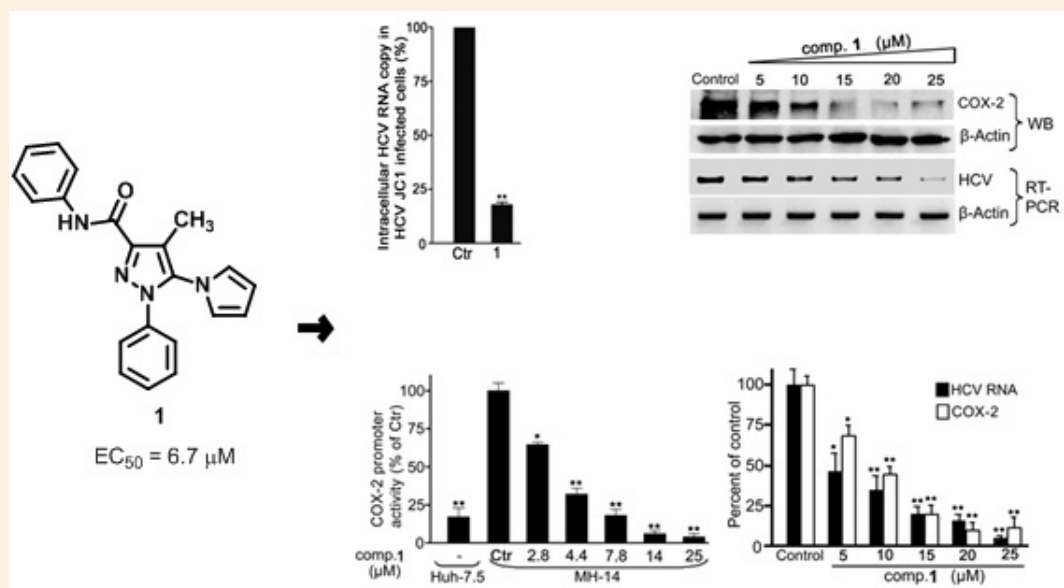
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Design and Synthesis of New 1-Phenyl-5-(1H-pyrrol-1-yl)-1H-pyrazole-3-carboxamides as anti-HCV agents targeting Cyclooxygenase-2

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Hepatitis C virus (HCV) is single-stranded (ss) RNA Hepacivirus in the Flaviviridae family. The current HCV therapy suffers from inadequate sustained viral response rate, rapid emergence of drug resistance, in particular for patients infected with genotype 1 HCV. (1,2) We synthesized new pyrazolecarboxamide derivatives (3) as anti-Hepatitis C virus agents, and investigated the mechanism of inhibition (Chart 1). The most active compounds inhibited the subgenomic HCV replicon 1b in Huh 5-2 cells with EC₅₀ of 6.7 μ M and selectivity index of 23 against HCV 1b. Hit compound 1 did not target HCV NS5B or HCV IRES mediated translation; evaluation of the mechanism of anti-HCV activity of 1 revealed that it suppressed HCV-induced COX-2 mRNA and protein expression, exhibiting an IC₅₀ of 3.2 μ M in COX-2 promoter-linked luciferase reporter assay. Our data suggest that the pyrazolecarboxamide derivatives behave as anti-HCV agents by targeting COX-2 at both the transcriptional and translational levels. These results provide a strong basis for hit optimization of this new chemical class of HCV inhibitors. (1) Tan, S. L., Pause, A. et al. Nat. Rev. Drug. Disc. 2002, 1, 867-881. (2) Barreca, M. L., Manfroni, G. et al. J. Med. Chem. 2013, 56, 2270-2282. (3) Manvar, D., Pelliccia, S. et al. Eur. J. Med. Chem. 2015, 90, 497-506



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Novel potent antiviral derivatives against poliovirus

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The Global Polio Eradication Initiative, established in 1988, has made substantial progress toward its target, with only 3 countries that have not yet eliminated wild poliovirus (PV).¹ More problematic for the eradication is the persistent infections that Sabin strains can establish in immune-deficient individuals (iDVPV), with evolution rates similar to those for wild virus and circulating vaccine-derived polioviruses (cVDPVs). Thus, iDVPVs must be considered a potential source for outbreaks and for reemergence of polio after eradication. Antiviral drugs offer a promising complementary or alternative approach to the use of vaccines to control poliovirus infections, particularly for chronic persistent infections in immunodeficient individuals during the final stages of eradication and for post-eradication reemergence.² WHO has recently stressed the importance of research on antivirals and recommended the development of antiviral drugs against PV as a tool to maintain polio-free status after global eradication.

We screened the in vitro antiviral activity of our in-house chemical library against PV Sabin strains and we found two hits, RC 304 and RC 305, active at submicromolar concentration against Sabin 2 and 3 strains. To increase the potency of these antiviral agents, we designed derivatives of these hits and identified antivirals that were also active against Sabin 1. These newly synthesized

compounds were highly active against the three PV Sabin reference strains and a large panel of wild and vaccine-derived PVs isolated from patients with acute flaccid paralysis or immune-deficient subjects. In particular, compounds RC 402 and RC 444 were active at nanomolar concentration against PV Sabin 1, 2, 3 serotypes. Time of addition experiments suggested that these compounds are active in an early stage of viral replication, possibly the uncoating.³

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Design, Synthesis and Anti-corona Virus Activity of a Series of Acyclic Fleximer Analogues

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Replication is intrinsically involved in the lifecycle of all viruses, thus their survival relies on DNA or RNA polymerases. One of the most effective strategies in targeting this enzyme is through the use of nucleos(t)ide analogues. An area in which these analogues have been barely explored is the coronaviruses (CoVs), especially Severe Acute Respiratory Syndrome-CoV and Middle East Respiratory Syndrome-CoV. Previously it was found that a “split base” guanosine analogue (Flex-GTP) developed in our laboratory not only served as a better substrate for, but also retained full potency against, binding site mutations in guanosine fucose pyrophosphorylase due to interactions with secondary amino acid residues. Flex-GTP also served as an inhibitor of S-adenosylhomocysteine hydrolase, an adenosine-metabolizing enzyme. These observations strongly indicate that flexibility in the nucleobase scaffold can be a powerful tool for developing drugs that can bind to atypical enzymes in biologically significant conformations. Based on this information we designed and synthesized a series of novel nucleoside analogues by combining our “fleximer” base modification with various modified sugars of FDA-approved antiviral nucleoside drugs, including Acyclovir. This led to potent biological activity against a number of targets, including CoVs, with EC₅₀ values ranging from 9–51 μM. This is one of the best activity profiles for a nucleoside against coronaviruses observed to date, and has inspired a second generation of analogues, with biological testing currently underway. The results will be presented herein.

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Synthesis and in vitro Assay of Deoxyhypusine Synthase Inhibitors as Potential Anti-HIV Agents

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HIV chemotherapy mainly focuses on parallel inhibition of several viral enzymes (cART). However, in order to reduce long-term toxicities and the development of (multi-)drug resistance, it is mandatory to identify new potential targets. One option are cellular cofactors that are essential for viral replication like the eukaryotic initiation factor 5A (eIF-5A). This protein acts as a cellular cofactor of the HIV Rev protein in the process of nucleocytoplasmic transport of incompletely-spliced and unspliced viral transcripts. Activation of eIF-5A involves a unique post-translational modification which is catalyzed by two enzymes, the deoxyhypusine synthase (DHS) and the deoxyhypusine hydroxylase (DOHH). Targeting the DHS efficiently suppresses the activation of eIF-5A leading to an inhibition of HIV replication, which has been shown by active compounds like the guanlylhydrazone CNI-1493 and analogues of the natural substrate, e.g. GC7.

Recently, we reported an *in silico* designed structurally different inhibitor containing an indole core fragment and amine/guanidino moieties that showed dose-dependent activity against DHS and HIV-1 *in vitro* without causing cytotoxic effects. This hit compound has been employed as a lead structure for further optimization of binding affinity and development of new potential drugs. Here we present the synthesis and biological evaluation of a second substance identified in the initial virtual screening and several new compounds with modifications regarding the substitution pattern, alkyl chain lengths and the aromatic scaffold. The synthesis is based on *in situ* C-C cross coupling and indole cyclisation or click chemistry as the key step to obtain 2,5- and 3,5-substituted indole derivatives as well as one 1,4-substituted triazole compound.

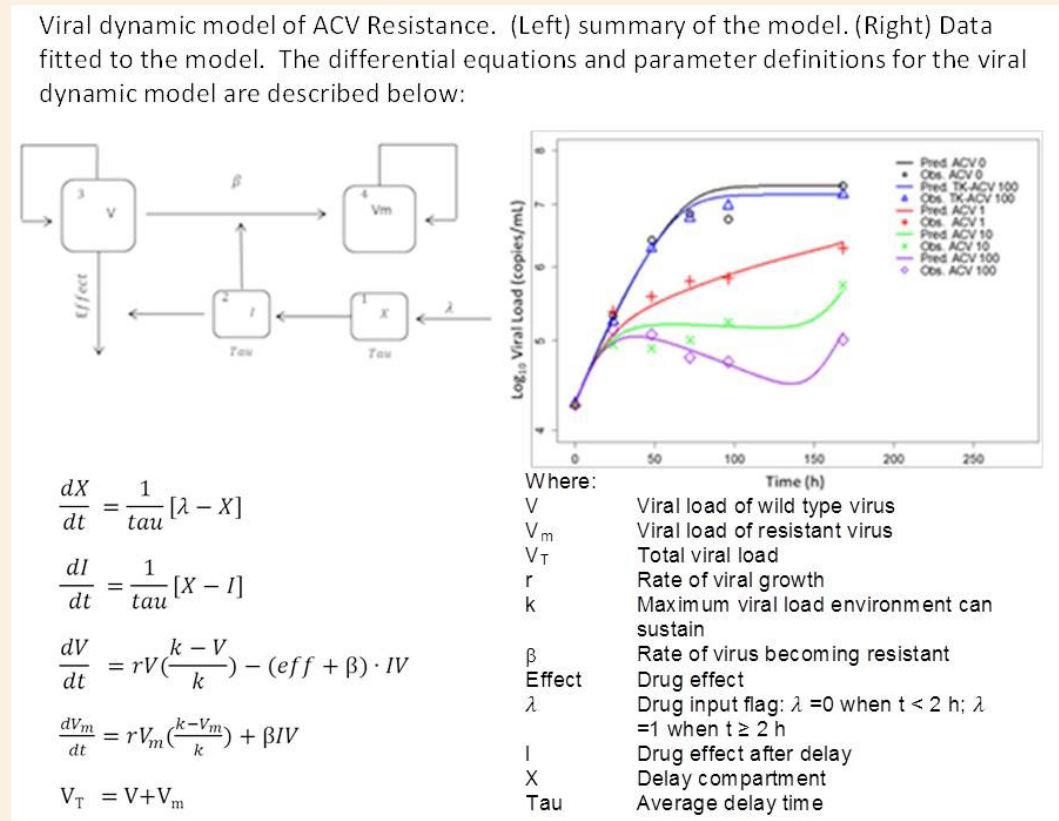
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Modeling the Evolution of Acyclovir Resistance with an In Vitro Compartment Model and Viral Dynamic Modeling

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Variants of herpes simplex virus (HSV) that are resistant to acyclovir (ACV) pre-exist at low frequencies in all laboratory and clinical isolates of HSV. Emergence of ACV resistance occurs as an outgrowth of resistant viruses already present in the infected individual in response to the selective pressure of the ACV. In healthy patients, the immune system limits the outgrowth of the resistant viruses, but this is less likely to occur in immune privileged sites of infection or when the function of the immune system is compromised. We developed an in vitro compartment model to recapitulate this process in cell culture and viral kinetic modeling to quantify changes in key kinetic parameters of emerging resistance. This model was validated by monitoring the evolution of ACV-resistance by quantifying viral DNA by qPCR, titrating infectious virus, and measuring the frequency of phenotypic resistance with a deep phenotyping assay. Resulting data were evaluated by viral dynamic modeling (see below). This powerful new approach has the potential to identify new strategies to prevent the emergence of resistance in high risk populations.



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Ryptophan Derivatives That Bind HIV Glycoproteins GP120 and GP41

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HIV-entry inhibitors may represent an advantage over the existing therapeutic approaches that target viral enzymes such as reverse transcriptase or protease, since they may avoid virus uptake by the target cells and show full efficacy against viruses resistant to the existing anti-HIV inhibitors.¹

The entry of HIV into its target cells is a complex, multi-step process involving attachment to host cell and CD4 binding, coreceptor binding and membrane fusion. All these three steps have been considered for the drug design of HIV-1 entry inhibitors. However, it might be preferable to develop anti-HIV drugs targeting viral proteins (gp120 and gp41) rather than host cell molecules (CD4, CCR5 and CXCR4), because binding these host cellular molecules might interfere with their normal functions, causing toxic or adverse effects.¹ Based on previous results of our group² novel compounds containing 6-18 tryptophan residues on their periphery have been prepared. These compounds significantly inhibit HIV replication by binding to gp120 and gp41. The results obtained so far indicate that 6-9 tryptophan residues on the periphery are sufficient for anti-HIV activity and associated gp120/gp41 binding. The synthesis, antiviral evaluation and SPR (Surface Plasmon Resonance)-based studies for these compounds will be presented.

ACKNOWLEDGMENTS: This work has been supported by the Spanish MINECO (project SAF2012-39760-C02) Plan Nacional de Cooperación Público-Privada. Subprograma INNACTO (project IPT-2012-0213-060000) the Comunidad de Madrid (BIPEDD2-CM-S2010/BMD-2457) and by "The Centers of Excellence" of the K.U.Leuven (EF-05/15 and PF-10/18). The Spanish MEC/MINECO are also acknowledged for a grant to E. Rivero-Buceta

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Limonoids from *Melia azedarach* fruits as inhibitors of Flaviviruses

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Melia azedarach, commonly known as chinaberry, is a species of the botanical family of the Meliaceae. It is native to the temperate regions of Asia, but is now naturalized in southern Europe, Africa and the United States, following the spread made by man; the main utility of chinaberry is its timber of high quality.

Melia azedarach exhibits a range of biological activities. Extracts from various parts of plant showed an interesting antifungal and antibiotic potential, activity against human and animal parasites, antioxidant properties. The lipophilic limonoids from *M. azedarach*, the typically active compounds of the plant, are described as significantly active against different cancer cell lines. On the contrary, its antiviral potential is poorly investigated.

In our previous studies we characterized four tirucallane-type triterpenes from the fruits of *M. azedarach*: 3- -tigloyl-melianol, melianone, 21- -acetoxymelianone and methyl kulonate. Here we report results of an antiviral screening of the four tirucallane-type triterpenes in cell-based assays against HIV-1 and representative members of several RNA and DNA virus families.

The 3- -tigloyl-melianol and melianone showed a very interesting activity (with EC₅₀ values in the range of 3-11 µM) against three important human pathogens of Flavivirus genus: Dengue virus (DENV), West Nile virus (WNV) and Yellow fever virus (YFV). Flaviviruses represent the most prevalent arthropod-borne viruses worldwide and their infections are continuously re-emerging. Studies are ongoing to determine their mode of action.

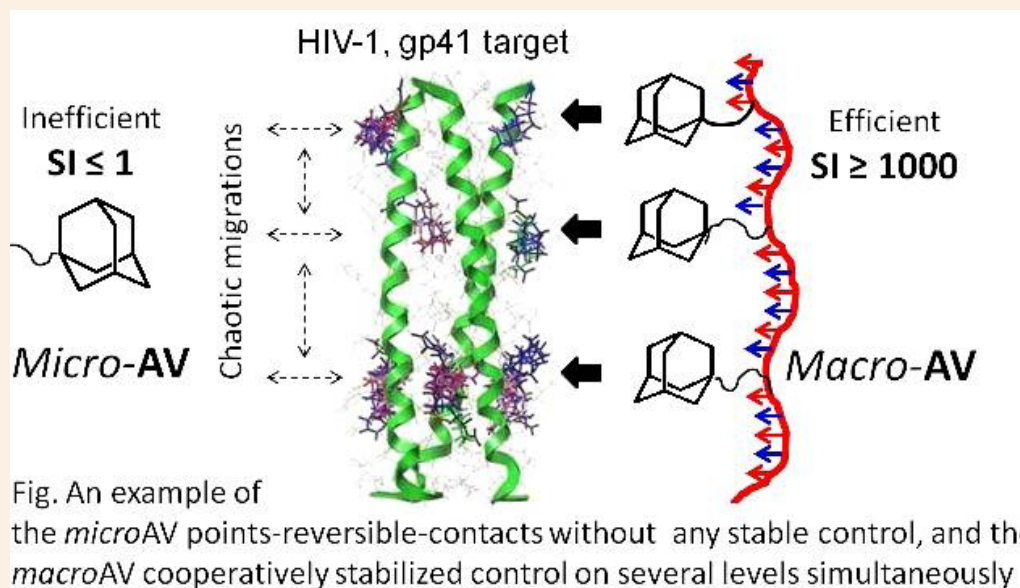
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Some Basic Principle for the Drug Resistance Preventive Antivirals Design

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The traditional small molecule-based antivirals can be fully efficient against the equivalently small targets: as anti-metabolites, or inhibitors of small-size active centers of enzymes/receptors. Within a viral life cycle such targets are located mainly inside an infected cell, where the viral replication starts and progresses. But extracellular virions, their entry, and intracellular assembly – new virions delivery are mediated by larger bio-macromolecules (and complexes) that cannot be stopped efficiently without an adequate counter-intervention. These targets have not a single but many regions for mutative adaptations and losing the drug control if the drug is small molecule that cover a one-point variable center only. As the result, the traditional small molecule antivirals (microAV) are incapable of blocking a viral drug resistance. The vaccine/antibody (Ab) applying therapy can be more efficient but Ab potency is also limited by the local region of antigen determinant. In theory, the probability (P) of a single one-point mutation (M) should be many orders more than probability of series of mutations (M1, M2, ... Mn) occurred in many mutative centers simultaneously: $P(M_i) \gg P(M_1M_2...M_n) = P(M_1)P(M_2)...P(M_n)$. Therefore, a viral drug-resistance formation could be suppressed fully when in addition to the microAV/Ab, the more large-scale macromolecular antivirals (macroAV) will be developed. These macroAV can be capable of multipoint blocking the numerous mutative-variable regions simultaneously (or step-by-step, from one to next target). This principle of antiviral activity was realized on basis of nano-competent macroAV evidently possessed an expanded antiviral protection against drug resistant strains and isolates of retro- (Fig.), orthomixo-, herpes- and other family viruses.



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5-(heteroaryl)-isoxazole: A new scaffold optimization as possible anti-HIV agent

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The recent explorations on heat shock protein were found interesting and appear promising target especially to overcome the drug resistance issue and latency in antiviral therapy. In continuation to our effort towards the discovery of possible antivirals, herein, we report a discovery of new scaffold based on N-substituted aryl-isoxazol as promising anti-HIV agents. A comprehensive structure activity relationship (SAR) was explored with eighteen newly synthesized compounds on the basis of anti-HIV activities and cytotoxicities studies. We found a preliminary compound 1 ($IC_{50}=238.8 \pm 8.38$ nM) with lower cytotoxicity ($CC_{50}=89.5 \pm 5.22$ μ M) and provided a new scaffold for anti-HIV drug development. Anti-HIV activity of compound 1 in TZM-bl (65.24%) and CEM-GFP (73.15%) cells at 1 μ M concentration as analyzed with luciferase assay and GFP quantitation respectively. The structural rationale and modeling studies supports the possibility for modulating HSP by this novel scaffold and effecting anti-HIV property. However, the biochemical characterizations are yet to be performed to elucidate its possible mode of action. (Authors thanks to DBT (BT/PR14237/MED/29/196/2010), India for financial support.)

93 Thiophosphoramidate Prodrugs: The Discovery of ALS-2200

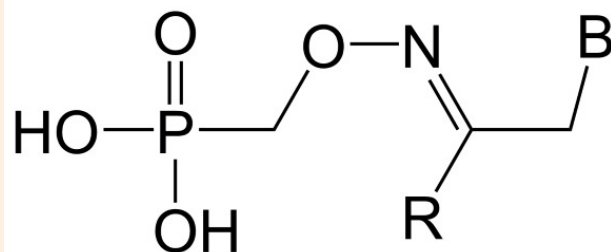
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Background: Nucleoside analogs play an important role in the treatment of viral diseases. In order to be effective as substrates for viral polymerases, nucleoside analogs must be phosphorylated inside cells to the triphosphate form. Reasoning that a more stable triphosphate form would provide a prolonged inhibition of viral replication, we investigated the possibility that a thiophosphoramidate could produce a long lived thiotriphosphate inside a cell. Applying this concept to the development of inhibitors of hepatitis C virus (HCV), two key questions were addressed: (i) would the HCV polymerase NS5B accept a thiotriphosphate as a substrate, and (ii) would a thiophosphoramidate activate to produce the thiotriphosphate. Herein we report the results of this investigation which led to the discovery of the thiophosphoramidate, ALS-2200, which subsequently demonstrated potent inhibition of HCV replication in patients with chronic hepatitis C (CHC). Methods: 2'-Methyl-uridine thiotriphosphate was prepared and evaluated for the ability to inhibit the HCV polymerase NS5B and serve as a chain terminator for further RNA elongation. Thiophosphoramidate prodrugs of 2'-Me-U, including ALS-2200, were prepared and assessed for inhibition in the cell based HCV replicon assay. Studies were conducted in Huh7 cells and human hepatocytes to determine the extent of conversion of ALS-2200 to the thiotriphosphate. The extent of thiotriphosphate formation in liver following oral dosing of ALS-2200 in dog was measured. Results and Conclusion: 2'-Me-5'-alpha-thio-UTP was found to be a good substrate and an efficient chain terminator of HCV polymerase, with an IC₅₀ = 0.22 microM. Thiophosphoramidate prodrugs of 2'-Me-U, as exemplified by ALS-2200, inhibited in the HCV replicon assay with an EC₅₀ of 0.15 microM. Following ALS-2200 exposure, high levels of thiotriphosphate were observed in both Huh7 cells and human hepatocytes, with the 2'-Me-5'-alpha-thio-UTP demonstrating a half-life of 37 hrs in human hepatocytes. High and sustained levels of thiotriphosphate were formed in dog liver following ALS-2200 dosing. These results are in agreement with the reduction in viral load associated with ALS-2200 treatment of CHC patients.

94 The design, synthesis and antiviral activity of new acyclic nucleoside phosphonates bearing unsaturated fragments in the chain

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More often than not, several viruses occur in a HIV-infected patient, e.g., over 80% of patients suffer from herpes and other virus-induced infections. It is important to search for substances with combined activity against different types of viruses. Herein, we report synthesis of new acyclic nucleoside phosphonate analogues bearing unsaturated fragments in the chain. One of the series is oxime-containing nucleoside phosphonates: 9-{2-[(phosphonomethyl)oximino]ethyl}adenine (1), -guanine (2) and 9-{2-[(phosphonomethyl)oximino]propyl}adenine (3). Oximes are rarely used in drug design and yet their conformational rigidity and their stability in hydrolytic tests may be useful for nucleoside side chain drug design. The compounds were synthesized using modified Mitsunobu procedure for key intermediate diethyl aminooxymethylphosphonate, and the efficient oxime forming "click" reaction was performed to form the target structures. A convenient procedure for aminoxy group detection was proposed. Products displayed moderate activity against HIV and herpes viruses in cell cultures and hepatitis virus C in replicon system without any toxicity up to 1000 µM. Other series of the compounds will be further discussed.



- 1** B = Ade; R = H
2 B = Gua; R = H
3 B = Ade; R = CH₃

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Effect of the Anti-Enteroviral Combination of Pleconaril, MDL-860 and Oxoglaucine Applied in Consecutive Alternating Administration (CAA) Course in Coxsackievirus B1 Neuroinfection in Mice

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There are many reasons to consider the chemotherapy as the main tool for control of enterovirus infections. At present, clinically effective anti-EV drugs do not exist. The main cause for this is the development of drug resistance. The monotherapy courses were the only approach used till now. For the first time in the antiviral investigations our team introduced the testing of combination effect of selective inhibitors of EV replication with different mode of action. In previous studies, we have proved the efficacy of the consecutive alternative administration (CAA) treatment course of the triple combinations of disoxaril/guanidine/oxoglaucine (DGO) and pleconaril/guanidine/oxoglaucine (PGO) in CVB1 infection in newborn mice. Drug sensitivity studies of viral brain isolates from mice, treated with DGO and PGO showed a preserved sensitivity to the drugs included in the combinations, i.e. prevention of the development of drug-resistance was registered.

We studied the effect of another triple combination by replacing guanidine.HCl with another enteroviral RNA inhibitor MDL-860 (DNB). The effect of the new combination PMO was tested in newborn mice infected s.c. with 20 LD₅₀ of CVB1 of CAA treatment course. The results obtained manifested an efficacy of PMO combination at comparatively narrow dose range of MDL-860: 50 - 75 mg/kg administered subcutaneously. It was found that the monotherapeutic course with MDL-860 (daily doses 25-100 mg/kg) showed a weak activity expressed only by a lengthening by 2.5 days of the mean survival time at 75 mg/kg. The simultaneous every day administration of the compounds in PMO was without effect. These data confirm the high efficacy of the CAA course approach and its perspectives for establishment of anti-enterovirus chemotherapy.

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Comparative study of the antiviral activity of broad-spectrum and enterovirus-specific inhibitors against clinical isolates of enterovirus-D68.

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Enterovirus-D68 was isolated for the first time in California in 1962 in children with pneumonia and bronchiolitis. Until 2008, the detection of EV-D68 infections has been very rare. Since 2008 EV-D68 got increased attention with different clusters of infections worldwide. In a recent epidemic in the United States, 1.153 people were confirmed with respiratory illness caused by EV-D68, mostly children with asthma or a history of wheezing. The virus also has been associated occasionally with polio-like illness (muscle weakness or paralysis). Moreover, EV-D68 was detected in specimens from 14 patients who died. EV-D68 thus emerged as a considerable global health threat, requiring the development of effective antiviral treatment. To assess the activity of antiviral molecules against EV-D68 a panel of 7 clinical isolates was selected consisting of representative strains of the three major genogroups. The following inhibitors were included in this study: (i) two capsid-binding compounds, pleconaril and pirodavir; (ii) the protease inhibitor rupintrivir; (iii) the host cell-targeting compound enviroxime; and (iv) the broad-spectrum antivirals favipiravir (T-705) and arbidol. Our preliminary results with the 7 clinical isolates reveal that pleconaril and rupintrivir efficiently inhibited *in vitro* EV-D68 replication. Favipiravir (recently approved in Japan to treat influenza virus infections) and enviroxime also resulted in a clear antiviral effect. On the other hand, pirodavir and arbidol resulted only in a modest inhibitory effect on EV-D68 replication. We will present data on the *in vitro* anti-EV-D68 activity of this compound-panel against the wider panel of recent EV-D68 isolates. LS is funded by the Chinese Scholarship Council. LD is funded by the Research Foundation of Flanders (FWO).

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Hydroxylated Tropolones as Lead Compounds for Novel Anti-Hepatitis B Virus Drugs

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Hepatitis B virus (HBV) chronically infects 350 million people world-wide, leading to about 700,000 deaths annually. Nucleos(t)ide analogs that target the viral reverse transcriptase control but rarely cure the infection, so more effective therapies are badly needed. The HBV ribonuclease H (RNaseH) is a logical drug target because it is the second of only two viral enzymes essential for viral replication, but it has not been targeted due to technical barriers. We recently established a novel low throughput screening pipeline for RNaseH inhibitors.

Fifty-two α -hydroxytropolone (HT) and related troponoid compounds were screened for anti-HBV RNaseH activity. Thirteen compounds had detectable activity against either genotype C or D HBV RNaseH in biochemical studies, with 5 inhibiting at ≤ 20 μ M in the primary screens. The best IC_{50} s were < 10 μ M. A low-resolution structure-activity relationship (SAR) was established that was consistent with the metal-chelating mechanism reported for the HTs against the HIV RNaseH. The SAR also implies that the HBV RNaseH active site is likely to be narrower than the HIV active site, which should facilitate optimization of efficacy and specificity. Six compounds inhibited viral replication in culture by blocking the viral RNaseH. EC_{50} values ranged from 0.34-9.1 μ M and CC_{50} s were 25-79 μ M. The best compound had an EC_{50} of 0.34 μ M and a therapeutic index of 94. Large differences were observed in the basal RNaseH activity among HBV a panel of patient-derived RNaseH variants, both within and between genotypes. However, no discernable differences were observed in sensitivity of the variants to α HTs. Potential additive or synergistic interactions between HTs and the approved nucleos(t)ide analogs are being assessed.

Therefore, HT compounds are promising candidates for development as novel anti-HBV drugs. Improving selectivity for the HBV enzyme without exacerbating toxicity will be key during chemical optimization. Anti-RNaseH drugs are envisioned to be used in combination with the nucleos(t)ide analogs to suppress HBV replication far enough to clear the virus in a greater proportion of patients.

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Preclinical Studies with Novel Herpesvirus Inhibitors from Two Chemical Classes

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Herpesvirus DNA replication requires several enzymes in the nucleotidyl transferase superfamily (NTS) that have recombinase and nuclease activities. We previously found that compounds which block NTS enzymes efficiently inhibit replication of herpes simplex virus (HSV)-1 and HSV-2 replication, and also replication of human cytomegalovirus. Compounds in several chemical classes reduced HSV replication by up to 1 million-fold at 5 μ M in Vero cells or primary human foreskin fibroblasts, and were often more effective than the approved anti-herpesvirus drug acyclovir (ACV). We have shown that a polyoxygenated heterocycle compound, ciclopirox, and a hydroxylated tropolone, β -thujaplicinol, profoundly inhibit replication of ACV-resistant mutants.

Here, we report that resistance evolves against NTS inhibitors ciclopirox and β -thujaplicinol much more slowly than against ACV. After 3 rounds of growth in the presence of ACV, 6 independent lineages of ACV-selected HSV-1 strain KOS were no longer inhibited by ACV ($10 \times EC_{50}$), whereas ACV treatment inhibited replication of the parental virus more than 1700-fold. In marked contrast, ciclopirox-selected viruses were inhibited only about 2-fold less well than the parental strain by ciclopirox, and sensitivity of β -thujaplicinol-selected viruses to β thujaplicinol remained unchanged. We also found that topical ocular treatment with ciclopirox or β -thujaplicinol protected mice from HSV-1 corneal infection as well as ACV, reducing weight loss and virus titer in the nervous system compared with a diluent control.

Thus, both ciclopirox and β -thujaplicinol apply a much higher barrier to evolution of viral resistance than does ACV, and both compounds are effective against HSV-1 infection *in vivo*. These properties, and their different mechanism of action than the nucleoside analogs, suggest that NTS inhibitors are promising candidates to be developed into highly effective treatments for herpesvirus infections. Such drugs could be used as monotherapies, in combination with existing drugs, or as salvage therapies for patients with drug-resistant herpesvirus infections.

99 Targeting protein-protein interactions as a successful therapeutic strategy against viral infectious diseases

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Protein-protein interactions (PPIs) are attractive targets for therapeutic intervention because of their crucial roles in several biological processes. Targeting these interactions with small molecule inhibitors is a research area of considerable interest in medicinal chemistry. Recently, a lot of information on PPIs in different viral life cycles has been made available through chemical biology experiments providing many opportunities in the fight against pandemic infectious diseases such as AIDS and influenza. [1-2] Herein, we report the application of computational strategies for the identification of novel antiviral inhibitors targeting PPIs. In detail, a structure-based virtual screening approach was applied with the aim of identifying novel HIV-1 entry inhibitors targeting the interaction between CD4 and HIV-1 gp120.[3] Four novel classes of inhibitor emerged that have significant anti-HIV-1 activities. Similarly, novel 2-aminothiazolones were synthesized and biologically tested in order to investigate their potential in inhibiting the HIV infection. Experiments demonstrated that the compounds acted as early inhibitors of the gp120-CD4 interaction.[4] On the other hand, we combined different computational techniques in order to identify new small molecule influenza A virus polymerase inhibitors targeting the PA-PB1 protein-protein interaction.[5-6] Some compounds possessing a 3-cyano-4,6-diphenyl-pyridine nucleus emerged as effective anti-influenza A agents.

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100 Rhodanine Derivatives as potent anti-HIV microbicides

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Preventing HIV transmission by the use of a vaginal microbicide is a topic of considerable interest in the ongoing fight against AIDS. [1] Both a potent anti-HIV agent and an efficient formulation are required to develop a successful microbicide. In this regard, we recently discovered a series of rhodanine derivatives [2] able to inhibit HIV-1 replication at nanomolar concentration. Because of the high affinity binding of these compounds to albumin, their activity decreased of several times (from 20 to 100) once they were pre-incubated with serum before the in vitro assay, thus preventing their use as oral drugs for HIV. Conversely, preliminary in vitro ADME studies highlighted important features, in addition to the anti-HIV activity profile, which make rhodanines promising candidates as anti-HIV microbicides: 1) excellent cell permeability; 2) stability higher than 90% at pH between 4 and 7; 3) acceptable solubility at pH 4.2. Furthermore, it has been shown that the complexity of sexual transmission of viral pathogens requires the identification of compounds able to block the early events during the cycle of viral infection. [3] Being able to inhibit the very early stage of HIV entry process, as demonstrated by a time of addition experiment, rhodanines are rather suitable for the use as microbicide. On this basis a vaginal gel formulation of the best anti-HIV candidate, MAS510, was developed and evaluated in vitro.

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101 New Antiviral Molecule From *Phyllocaulis Boraceiensis* Mucus to Treat Measles

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Measles is a viral disease with respiratory transmission and is highly contagious. Despite being controlled by vaccination, currently has registered numerous cases in various areas of the world. Terrestrial gastropods exude mucus by the body surface, when traveling, to protect its body from mechanical injury, desiccation or contact with harmful substances. Mucus of mollusks has been studied as a source of new natural compounds with diverse biological activities. In order to identify, isolate, purify and sequence molecules present in the mucus of the land slug *P. boraceiensis* with antiviral action “in vitro” were used fragmentation by chromatography and mass spectrometry in order to determine the active molecules and assay of biological activity, qPCR and Immunofluorescence labelling to determine the biological activity. The viral activity was determined “in vitro” using Vero cells infected with measles virus. The crude sample and four fractions were tested in cultures infected with measles virus and verified the presence of a molecule in mucus with antiviral action, either by determination of cytopathic effect in cell cultures infected either by immunofluorescence or RT-PCR. Antiviral assay in plates allowed a reduction in growth of measles virus in 64-fold and protection rate, measured by qPCR, was at over 80% in Vero cells infected with measles and treated with fraction 39. This fraction was identified as a mixture of unsaturated carboxylic acids.

102 Selective inhibition of HBV replication by novel neplanocin A derivatives

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Hepatitis B virus (HBV) is a major cause of serious liver diseases, such as cirrhosis and hepatocellular carcinoma. Chronic HBV infection is currently treated with nucleoside analogs, such as lamivudine, entecavir, and tenofovir. They are potent inhibitors of HBV DNA polymerase, which also functions as reverse transcriptase. Although these analogs are effective in HBV-infected patients, emergence of drug-resistant mutants and viral reactivation after treatment interruption are major concerns in antiviral chemotherapy against HBV. Therefore, it seems still mandatory to identify and develop novel inhibitors of HBV. We evaluated several neplanocin A derivatives for their inhibitory effect on HBV replication in HepG2.2.15.7 cells, a HepG2.2.15 clone producing a higher amount of viral particles than the parental cells. Among the neplanocin A derivatives, (1*S*,2*R*,5*R*)-5-(5-bromo-4-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (AR-II-04-26) and (1*S*,2*R*,5*R*)-5-(4-amino-3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)-3-(hydroxymethyl)cyclopent-3-ene-1,2-diol (MK-III-02-03) were found to be selective inhibitors of HBV replication. The EC₅₀ and CC₅₀ of AR-II-04-26 were 0.77 ± 0.23 and > 100 μM, respectively. On the other hand, the EC₅₀ and CC₅₀ of MK-III-02-03 were 0.88 ± 0.43 and 67.8 ± 7.7 μM, respectively. Interestingly, AR-II-04-26 and MK-III-02-03 affected HBsAg levels in culture supernatants of HepG2.2.15.7 cells, but lamivudine did not. In addition, unlike neplanocin A, both compounds were not inhibitory to the activity of S-adenosyl-L-homocysteine hydrolase. Therefore, it appears that the mechanism of action of AR-II-04-26 and MK-III-02-03 differs from that of lamivudine. Although their exact target molecule remains unknown, AR-II-04-26 and MK-III-02-03 are considered as promising leads of novel HBV inhibitors. Further studies are in progress to optimize their chemical structures.

103 Influenza virus and RSV ViroSpot™ Assays for high-throughput virology testing

Carel van Baalen¹, Juthatip Keawcharoen¹, Leon de Waal¹, Germaine Penders¹, Tamara Roelofse¹, Ella Nirmala², Rik de Swart², Guus Rimmelzwaan²

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Large-scale sample analyses in phase II and III clinical trials of novel antiviral compounds and vaccines require objective and reliable methods for high-throughput virus detection, quantification and characterization. Compared to molecular readouts, this is generally more difficult to achieve for methods which assess infectious virus titers, virus neutralization or virus phenotypes, including drug-resistance.

Here we developed ViroSpot assays, which combine classic virus culture techniques with automated sensitive detection of immunostained virus-infected cells in 96-well microtiter plates. Counting individual infected cells, plaques or infected areas in 96-well plates provided values that were directly proportional to virus propagation. Incubation periods for virus titration, virus neutralization and inhibition assays were significantly reduced compared to conventional techniques, while traceability of raw data and objectivity of results improved. Propagation of resistant minority species in presence of selective inhibitors was readily detected. Because solidifying reagents, such as agarose, avicel or carboxymethyl cellulose, were not necessary, cognate virus yields could be stored for genotyping and further phenotypic analyses. The sensitivity of detection at the single cell level omitted the need for prior virus amplification in absence of inhibitors. This facilitated direct phenotypic analyses of heterogeneous virus populations in clinical specimens.

In conclusion, the ViroSpot methodology described here offers novel possibilities for phenotypic resistance monitoring, and for standardization and upscaling of influenza virus and RSV culture-based readouts in clinical trials.

104 Identification and Synthesis of 1,4-Disubstituted Piperidines as New Entry Inhibitors Of H1N1 Influenza Virus

Sonsoles Velázquez¹, Sonia de Castro¹, Evelien Vanderlinden², Lieve Naesens², María José Camarasa¹

¹Instituto de Química Médica-CSIC, Madrid, Spain, ²Rega Institute for Medical Research KU Leuven, Leuven, Belgium

The recent outbreaks of the highly pathogenic avian A/H5N1 and pandemic A/H1N1/2009 influenza viruses have emphasized the need for anti-influenza drug discovery. The piperidine nucleus is an attractive drug template exploited in agents with applications as anti-histaminic, anti-inflammatory, fungicidal, bactericidal, anticancer, analgesic, CNS stimulant and or anti-depressant activities. In an effort to discover novel compounds as potential anti-influenza drugs, several molecules of our diverse in-house library were screened for anti-influenza virus activity. Following this approach, we identified disubstituted piperidine-based compounds as interesting hit compounds that display low micromolar activity against the influenza A/PR/8/34 virus (A/H1N1) in cell culture. To investigate the structure-activity relationships, several analogues were easily synthesized by a one-step Ugi four-component reaction from an amine, an isocyanide, N-substituted piperidones and carboxylic acid components. Time-of-addition studies showed that the compounds act during influenza virus entry. Mechanistic studies are ongoing to precisely identify their antiviral target, and explain the basis for the observed H1N1 specificity.

105 Dengue virus (DENV) serotype and endothelial cell type/origin determine endothelial dysfunction after DENV infection.

Peter Vervaeke, Sam Noppen, Sandra Liekens

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

Dengue is the most prevalent arthropod-borne viral disease in humans. Four genetically related serotypes of dengue virus (DENV 1-4) are transmitted to humans by *Aedes* mosquitoes in (sub)tropical areas and cause an acute febrile illness, dengue fever (DF), or the potentially life-threatening dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS), which are characterized by increased vascular permeability, plasma leakage and circulatory failure. The pathogenesis of DHF/DSS is poorly understood and, although endothelial cells (ECs) represent the primary fluid barrier of the blood vessels, the extent to which these cells contribute to DENV pathology is still under debate. *In vitro*, ECs are permissive for DENV infection and replication. However, the use of different serotypes and EC types makes it difficult to compare studies and may be responsible for the contradictory results regarding DENV virulence and endothelial dysfunction after DENV infection of ECs.

We have compared virus infectivity and EC responses upon infection of microvascular (HMVEC-d, HMEC-1) and macrovascular (HUVEC) ECs with DENV 1-4. DENV-4 proved to be the most infectious serotype, followed by DENV-2 and -1, while DENV-3 infection resulted in lower infection rates. The microvascular cell line HMEC-1 was the most permissive cell type for DENV 1-4, whereas the infection rates of the primary cells (HUVEC and HMVEC-d) were lower but comparable. The maximum infection rate correlated with the cellular expression level of heparan sulfate, which acts as receptor for DENV on ECs. A Bio-Plex assay, designed to quantify the levels of 17 cytokines, known to regulate endothelial cell permeability or immune cell function, showed that the cytokine expression level does not strictly correlate with infection rate and that different serotypes and cell types result in specific cytokine expression patterns. Annexin V-FITC/propidium iodide staining demonstrated a time-dependent induction of apoptosis, which correlated with the degree of infection. These results indicate that DENV infection of ECs and DENV-induced cytokine secretion and apoptosis are highly dependent upon the EC origin and DENV serotype. Experiments are ongoing to investigate vascular permeability in the infected ECs.

106 Stereoselective synthesis of iso-carbocyclic nucleoside analogues

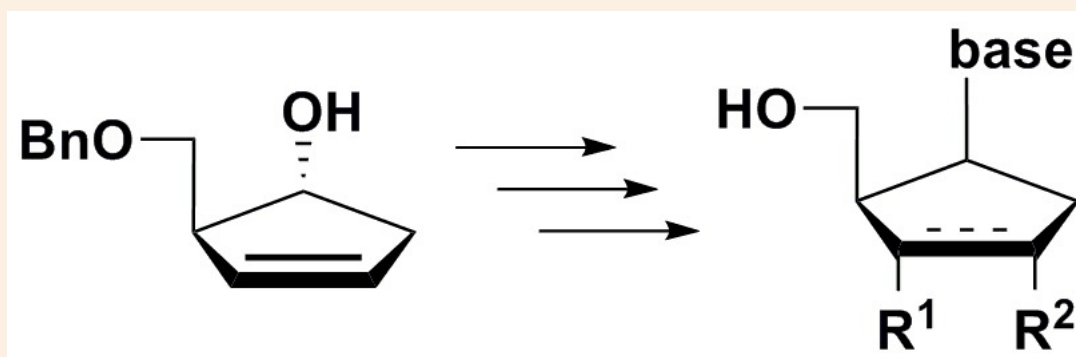
Simon Weising, Chris Meier

University of Hamburg, Hamburg, Germany

In the past few decades carbocyclic nucleosides were synthesized which have interesting antiviral properties. In comparison to their natural counterparts the carbocyclic nucleosides often show higher hydrolytic and enzymatic stability and lower cytotoxicity. [1] The carbocyclic nucleosides abacavir and entecavir are used in antiviral therapy against the HI-virus and the hepatitis B virus, respectively.

The nucleoside analogue *carba*-dT has been shown to be a potent inhibitor of HIV's reverse transcriptase. In addition, the 1,2-*cis*-substituted analogue *carba-iso*-dT has shown a 20-fold decrease of activity without recognizable cytotoxicity.[2]

On the basis of these results we synthesized new *carba-iso*-nucleosides with different modifications in the cyclopentane-scaffold. The syntheses of the *carba-iso*-deoxynucleosides started with the protection and hydroboration of enantiomerically pure (1*S*,2*R*)-2-(benzyloxymethyl)cyclopent-3-enol.[3] Afterwards, a Mitsunobu coupling with *N*3-protected pyrimidines and 6-chloropurines, respectively, followed by deprotection was performed to obtain the target compounds. In another approach the nucleobases were directly condensed with the enantiomerically pure (1*R*,2*S*)-2-(benzyloxymethyl)cyclopent-3-enol to synthesize a variety of nucleosides. With this approach it was possible to obtain several *carba-iso*-didehydro-, *carba-iso*-dideoxy- and *carba-iso*-ribonucleosides. [1] C. Desgranges, G. Razaka, M. Rabaud, H. Bricaud, J. Balzarini, E. De Clercq, *Biochem. Pharmacol.* 1983, *32*, 3583-3590. [2] P. L. Boyer, B. C. Vu, Z. Ambrose, J. G. Julias, S. Warnecke, C. Liao, C. Meier, V. E. Marquez, S. H. Hughes, *J. Med. Chem.* 2009, *52*, 5356-5364. [3] O. L. Ludek, T. Krämer, J. Balzarini, C. Meier, *Synthesis* 2006, *8*, 1313-1324.



107 Efficient Synthesis of Ribavirin-Phosphoramidites for Biochemical Applications

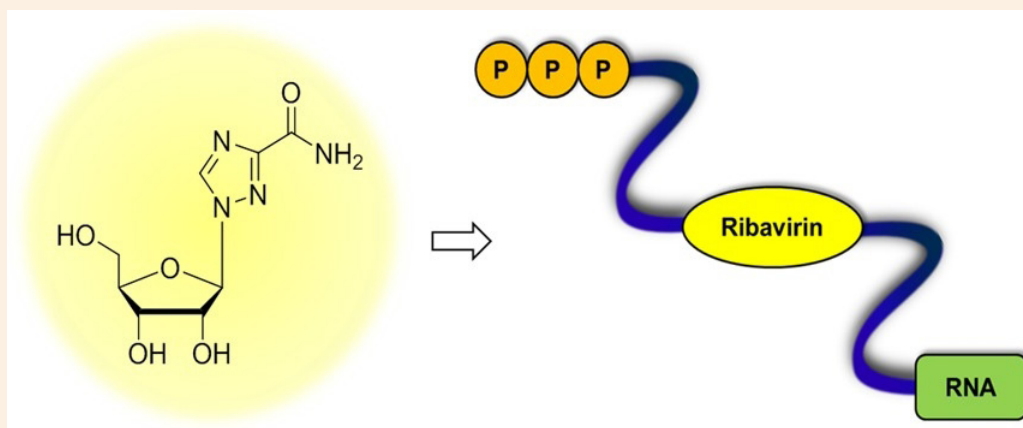
Matthias Winkler¹, Ivo Sarac¹, Cristina Ferrer-Orta², Núria Verdaguer², Chris Meier¹

¹University of Hamburg, Hamburg, Germany, ²Molecular Biology Institute of Barcelona, Barcelona, Spain

The nucleoside analogue ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) and pegylated interferon are still used as standard treatment of care for patients with chronic hepatitis C. Due to its broad antiviral activity against several DNA and RNA viruses, ribavirin can be used as a model substrate for diverse biological applications. Here we report the solid phase synthesis of different RNA oligonucleotides containing the broad-spectrum antiviral ribavirin. Sequence-specific incorporation of ribavirin into oligonucleotides enables their use in elongation assays, co-crystallization with viral polymerase, e.g. picorna virus polymerase and further biochemical experiments.

In order to synthesize the modified oligonucleotides the corresponding ribavirin-phosphoramidite was needed. Starting from ribavirin, the 2'-O-TBDMS protected phosphoramidite was synthesized in six steps. Additionally, we synthesized the 2'-O-TC-phosphoramidite in seven steps, respectively. Both phosphoramidites were obtained in excellent overall yields. The phosphoramidites were used for the synthesis of four specific oligonucleotide sequences, which are currently evaluated as probes for the crystallization of viral polymerases of the *picornaviridae* family.

Furthermore, we applied our recently developed method for the preparation of DNA- and RNA 5'-triphosphates to an oligonucleotide bearing ribavirin. RNA triphosphates are involved in various important biological interactions and can therefore serve as interesting substrates for a wide field of possible applications.



108 Development and application of pseudotype virus for antiviral drug screening against highly pathogenic avian influenza virus (HPAI) under biosafety level 2 (BSL2) facility

Hwajung Yi, Junhyung Cho, Kisoong Kim

Division of Influenza Virus, Center for Infectious Disease, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju-si, South Korea

Influenza has been threatening the public health such as avian influenza virus human infection and pandemic flu in 2003 and 2009, respectively. Once the virus emerged into human population, public health response strategy has been focused to mitigate viral diseases through therapeutics and/or prophylactic approach. Majorities of candidate viruses with pandemic potency are likely highly pathogenic so that practical biosafety should be concerned with the first priority to handle certain viruses. In accordance with biological safety regulation, the biosafety level 3 (BSL-3) facility should be equipped to address highly pathogenic avian influenza (HPAI) virus and this hampered research and development of corresponding antiviral agents and vaccines. To attempt get over this hurdle, a pseudo-type influenza virus (PIV) having surface-glycoproteins (hemagglutinin and neuraminidase) from an highly pathogenic avian influenza strain was prepared and successfully employed in drug discovering assay at the level 2 biosafety (BSL-2) facility. Measuring infectivity of the PIVs into cells was accomplished by introducing a reporter gene (luciferase) to the PIV for visualization. Tens of hundreds of antiviral drug candidates were subjected to screen massively for antiviral efficacy under BSL-2 with PIV mimicking avian-influenza viral entry. Specific reductions of relative luciferase unit (RLU) with statistically significance were recruited as anti-influenza activities and these hits were applied for further investigation of inhibitory effects using wild-type viruses challenge in an experimental animal model. The pseudotype system developed in this study will facilitate the exploration of antiviral drug screening against influenza viruses, circumventing the need for high-level biosafety containment. This work was supported by intramural fund (#2013-NG43001-00) of the NIH, Korea.

109 **In vitro antiviral activity of *Scutellaria baicalensis* against dengue virus**

Keivan Zandi¹, Tong-Hye Lim², Nor-Aziyah Rahim¹, Meng-Hooi Shu¹, Boon-Teong Teoh¹, Sing-Sin Sam¹, Kim-Kee Tan¹, Sazaly Abubakar¹

¹Tropical Infectious Disease Research and Education Center (TIDREC), Department of Medical Microbiology, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia, ²Herbitech Sendirian Berhad, G-3-7, Plaza Damas Jalan Sri Hartamas, Sri Hartamas, Kuala Lumpur, Malaysia., Kuala Lumpur, Malaysia

Scutellaria baicalensis (*S. baicalensis*) is one of the traditional Chinese medicinal herbs that have been shown to possess many health benefits. In the present study, we evaluated the *in vitro* antiviral activity of aqueous extract of the roots of *S. baicalensis* against all the four dengue virus (DENV) serotypes. Aqueous extract of *S. baicalensis* was prepared by microwave energy steam evaporation method (MEGHETM), and the anti-dengue virus replication activity was evaluated using the foci forming unit reduction assay (FFURA) in Vero cells. Quantitative real-time polymerase chain reaction (qRT-PCR) assay was used to determine the actual dengue virus RNA copy number. The presence of baicalein, a flavonoid known to inhibit dengue virus replication was determined by mass spectrometry.

The IC₅₀ values for the *S. baicalensis* extract on Vero cells following DENV adsorption ranged from 86.59 to 95.19 µg/mL for the different DENV serotypes. The extract showed potent direct virucidal activity against extracellular infectious virus particles with IC₅₀ that ranged from 74.33 to 95.83 µg/mL for all DENV serotypes. The concentration of baicalein in the *S. baicalensis* extract was ~1% (1.03 µg/gm dried extract). Our study demonstrates the *in vitro* anti-dengue virus replication property of *S. baicalensis* against all the four DENV serotypes investigated. The extract reduced DENV infectivity and replication in Vero cells. The extract was rich in baicalein, and could be considered for potential development of anti-DENV therapeutics.

110 **NOVEL FLUTIMIDE ANALOGUES TARGETING THE INFLUENZA VIRUS PA ENDONUCLEASE**

Grigoris Zoidis¹, Erofilii Giannakopoulou¹, Annelies Stevaert², Vassilios Myrianthopoulos¹, Emmanuel Mikros¹, Lieve Naesens²

¹Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Athens, Greece, ²Rega Institute, Katholieke Universiteit Leuven, Leuven, Belgium

Influenza is a highly contagious infectious disease that affects millions of people every year. In the twentieth century, influenza caused more fatalities in Europe than any other infectious disease. Current vaccines against influenza virus require annual updating due to continuous variation of the viral antigens. Thus, anti-influenza virus drugs are vital as a first line of defense. At present, two classes of antivirals are available: the neuraminidase inhibitors oseltamivir and zanamivir, and the M2 proton channel blockers amantadine and rimantadine. For both drug classes, viral resistance is an emerging concern and, hence, novel antiviral agents with an alternative mode of action and favorable resistance profile are urgently needed. A promising new target is the influenza virus PA endonuclease, which performs the 'cap-snatching' reaction during viral mRNA synthesis, and is essential for virus replication.

This work aimed at the development of lead compounds by investigating the structural and stereoelectronic requirements for optimal influenza virus PA endonuclease inhibition.

The crystal structure of the N-terminal part of PA (PA-Nter) containing the catalytic endonuclease domain, was recently revealed, enabling structure-based development of PA inhibitors. In this study, we synthesized and evaluated several analogues of the natural compound Flutimide (a fungus-derived influenza virus endonuclease inhibitor with a 2,6-diketo-3-piperazine motif).

Using an enzymatic PA endonuclease activity assay, we show that these compounds block the enzymatic activity of PA-Nter. Theoretical calculations were also undertaken for providing a structural rationale for the interaction between the synthesized analogues and the viral protein and a number of molecular interactions contributing to binding affinity and specificity were elucidated. Theoretical results are supported by biochemical analyses of the enzymatic activity inhibition.

Overall, data presented reveal exciting strategies for the design and optimization of novel influenza virus inhibitors that target the viral PA endonuclease.

111 Novel phenyl(2-(phenylamino)pyrimidin-4-yl)methanones as potent non-nucleoside reverse transcriptase inhibitors

Petr Šimon¹, Ondřej Baszczyński¹, David Šaman¹, Gina Bahador², George Stepan², Eric Hu², Eric Lansdon², Petr Jansa²
¹IOCB, Prague, Czech Republic, ²Gilead Sciences, Inc, Foster City, CA, USA

A series of novel phenyl(2-(phenylamino)pyrimidin-4-yl)methanones (Fig. 1), as potential non-nucleoside reverse transcriptase inhibitors (NNRTIs) derived from diarylpyrimidine (DAPy),¹ was prepared by new synthetic approach. The pyrimidine ring was substituted with 4-cyanophenylamino moiety at C-2 position and with another aromatic system at C-4 position linked through the carbonyl linker. Further modifications are present at the C-5 position of the pyrimidine moiety. Structure and anti-HIV activity relationship (SAR) study was performed on a series of 23 compounds. The most potent derivative from the series exhibited low nanomolar anti-HIV activity ($EC_{50} = 4 \text{ nM}$) with no significant toxicity ($CC_{50} > 57.1 \text{ } \mu\text{M}$).

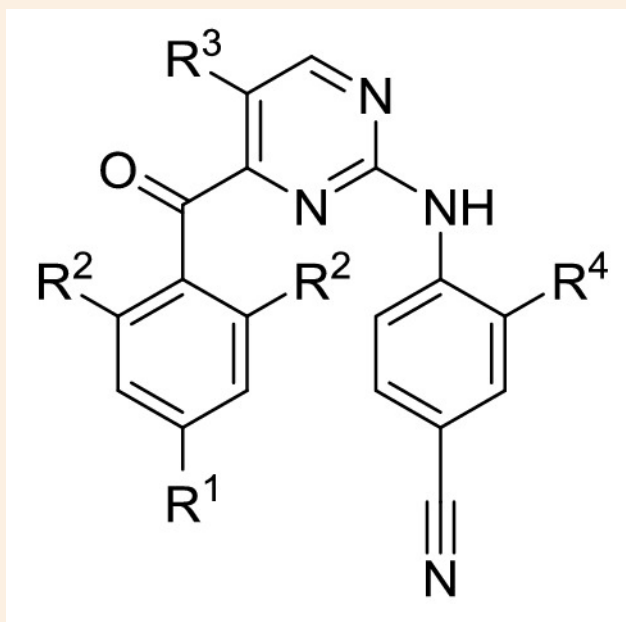


Figure 1. General structure of novel phenyl(2-(phenylamino)pyrimidin-4-yl)methanone derivatives studied.

ACKNOWLEDGMENTS. This work was supported by the subvention for development of research organization (Institute of Organic Chemistry and Biochemistry AS CR, RVO 61388963) and by Gilead Sciences.

LITERATURE:

1) Zhan, P.; Chen, X.; Li, D.; Fang, Z.; De Clercq, E.; Liu, X. *Med. Res. Rev.* 2013, 33 (S1), E1-E71.

Antiviral Chemistry

Chairs: **Chris Meier, Ph.D. and Katherine Seley-Radtke, Ph.D.**

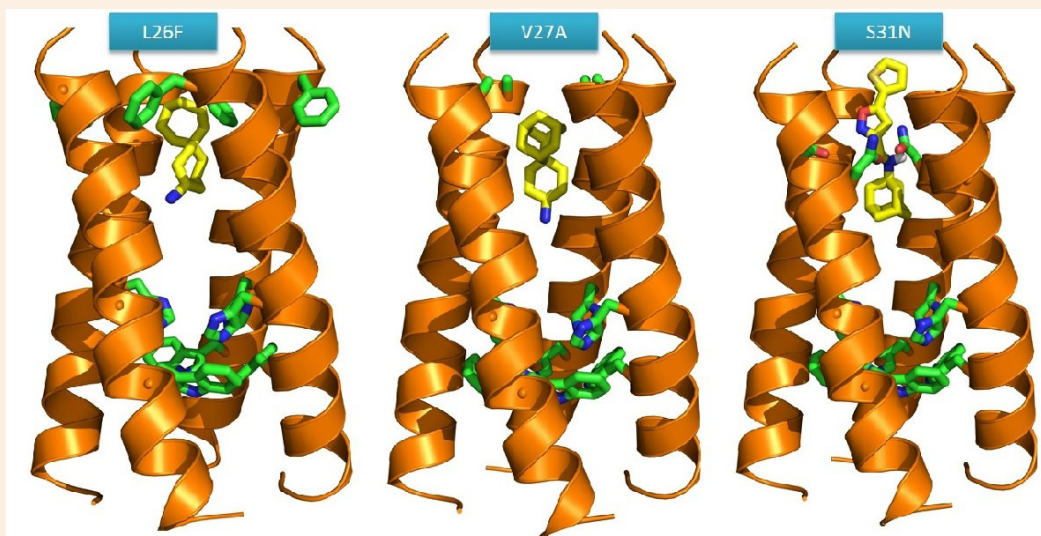
9:15 AM – 11:30 AM

ESTENSI – MEDICI

113 Design and pharmacological characterization of novel channel blockers targeting the drug-resistant M2 proton channels from the influenza A viruses

Jun Wang, Chunlong Ma, Fang Li
University of Arizona, Tucson, USA

Influenza A viruses are severe pathogens to public human health and approximately 10-20 % of the world population are infected with influenza A viruses in each year's flu season. However, we are limited in the countermeasures to fight against influenza infection, and oseltamivir is the only orally bioavailable drug in the United States. To meet the demand of novel antivirals that are active against drug-resistant influenza strains, we focus on the M2 proton channel from the influenza A viruses. Amantadine and rimantadine are channel blockers of the wild-type M2, but are no longer recommended to use in the U.S. due to prevalent drug resistance. Unlike other viral proteins, there are only three predominant drug-resistant M2 mutants, namely S31N, V27A, and L26F that confer both drug resistance and fitness of viral replication. Guided by molecular dynamics simulations, NMR and X-ray crystallography studies of M2, we have succeed in designing novel channel blockers against all three drug-resistant mutants. Their mechanism of action and antiviral activity were profiled in two-electrode voltage clamp assay, NMR, and plaque reduction assays, respectively. The therapeutic potential of S31N inhibitors were further characterized in terms of their potency against clinic isolate influenza strains, cytotoxicity, and genetic-barrier of drug resistance. The S31N inhibitors were found to display high potency against clinic isolate influenza strains which are resistant to amantadine or oseltamivir or both. Encouragingly, unlike amantadine, drug-resistant mutants were not readily emerged under the drug selection pressure of S31N inhibitors. Thus, S31N inhibitors are highly promising as drug candidates for the next generation of anti-influenza therapeutics.

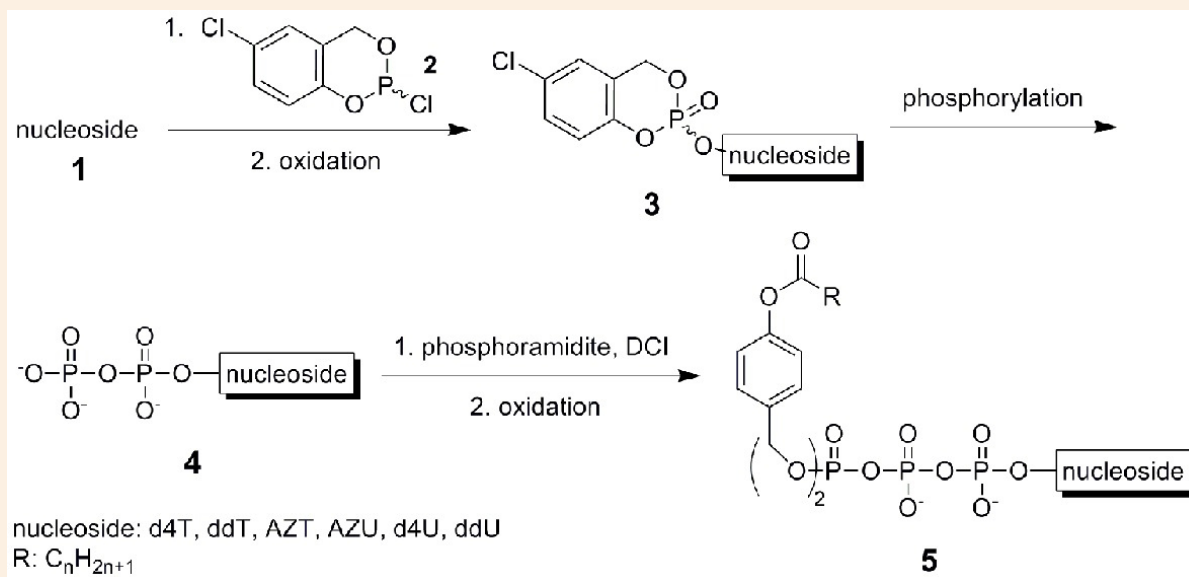


114 TRIPHOSPHATE PRODRUGS (TRIPPPRO'S) OF BIOLOGICALLY ACTIVE NUCLEOSIDES

Chris Meier¹, Tristan Gollnest¹, Tobias Nack¹, Dominique Schols², Jan Balzarini²

¹Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany, ²Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

Over the last decades a variety of nucleoside analogues were applied in antiviral chemotherapy. However, in some cases the antiviral potency of the nucleoside analogues, e.g. 2',3'-dideoxy- or 3'-modified-compounds, is limited due to the lack of efficient intracellular phosphorylation into their triphosphorylated form by host cell kinases. This problem cannot be solved by using the phosphorylated nucleosides because of their very high polarity. An option to overcome this problem is the use of masked nucleotide analogues, which are able to pass the cell membrane and deliver the corresponding nucleotides intracellularly by e.g. chemical or enzymatic hydrolysis. The cycloSal-prodrug system was developed for nucleoside monophosphates and was also applied to different nucleoside analogs. Recently, we reported on a first successful approach for the delivery of nucleoside diphosphates called DiPPro-approach. Here, we extended our work to the development of nucleoside triphosphate prodrugs. So far, no example of such a prodrug system has been reported. A number of d4TTP prodrugs with different aliphatic masking units have been synthesized via two different routes based on phosphoramidite or H-phosphonate chemistry. In addition, a variety of nucleoside analogues have been investigated. The target prodrug compounds **5** were obtained in yields up to 66%. Chemical hydrolysis studies, enzymatic cleavage in CEM/O cell extract, primer extension assays, PCR assays, CEM whole-cell incubations and antiviral HIV tests proved the successful delivery of NTP.



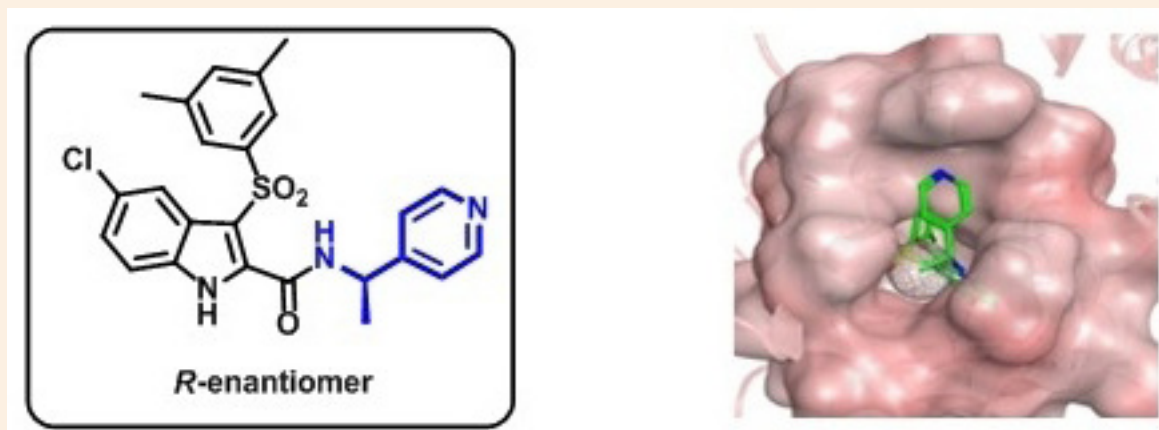
115 Indolylarylsulfones carrying a heterocyclic tail as very potent and broad spectrum HIV-1 non-nucleoside reverse transcriptase inhibitors

Valeria Famigliani¹, Giuseppe La Regina¹, Antonio Coluccia¹, Andrea Brancale², José A. Esté³, Romano Silvestri¹

¹Sapienza University of Rome, Rome, Italy, ²Cardiff University, Cardiff, United Kingdom, ³Universitat Autònoma de Barcelona, Badalona, Spain

HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key drugs of highly active antiretroviral therapy (HAART) in the clinical management of AIDS/HIV-1 infection. Our recent studies showed that indolylarylsulfones (IASs) bearing a cyclic moiety at the 2-carboxamide nitrogen linked through a short spacer group were endowed with potent antiretroviral activity.(1,2) Based on the results previously obtained, we aimed to expand the SAR studies by the introduction of new aryl or heteroaryl portions to the indole nucleus. Interestingly, for the first time IASs endowed with chiral centre have shown significant differences in term of antiretroviral potency. In particular, the R-enantiomer proved to be exceptionally potent and uniformly superior to the S-enantiomer against the whole viral panel. Docking studies showed that the methyl group of the R-enantiomer (Figure 1) pointed toward the cleft created by the K103N mutation, differently from the corresponding group of (S) counterpart. By calculating the solvent accessible surface, we observed that the exposed area of the RT in complex with S-enantiomer was larger than the area of the (R) complex.(3) 1) La Regina, G., Coluccia A. et al. J. Med. Chem. 2012, 55, 6634–6638. 2) Famigliani, V., La Regina, G. et al. Eur. J. Med. Chem. 2014, 80, 101-111. 3) Famigliani, V., La Regina, G. et al. J. Med. Chem. 2014, accepted.

Figure 1.



116 Molecular Modelling Studies on the Ternary Complex of Dengue Virus Polymerase.

Cecilia M. Cima, Salvatore Ferla, Joachim J. Bugert, Andrea Brancale
Cardiff University, Cardiff, United Kingdom

Dengue virus (DENV), an emerging mosquito-borne virus, is one of the most significant human pathogen among the genus *flavivirus* (family *Flaviviridae*). The four serotypes of DENV have a wide distribution and the disease is endemic in over 100 countries throughout South East Asia, Western Pacific, Africa and the Americas. The risk of infection affects almost half of the world population, accounting for 2.5 billion of people. Additionally, the dramatic increase in the incidence and the rapid spreading of the virus to new areas have recently raised even greater concern within the scientific community. The clinical manifestations may vary from a self-limiting illness, named dengue fever, to more serious conditions, such as dengue haemorrhagic fever and dengue shock syndrome. To date, the development of a vaccine has proven to be very challenging and specific antiviral drugs are not available. Among the ten viral proteins encoded by DENV genome, the RNA-dependent RNA polymerase (RdRp) represents one of the most attractive targets for the development of direct acting antiviral agents. Crystal structures for DENV-RdRp have been reported, however the polymerase domain in complex with the template and the nascent RNA strands (ternary complex) has not been crystallised. Molecular modelling techniques allowed us to create a model for the ternary complex structure of the polymerase. The hepatitis C virus (HCV) initiation complex was used as a template for the construction of the DENV polymerase active site and the model was refined through a series of molecular dynamics (MD) simulations. The results obtained from these calculations will be presented. These studies will provide important insights on the conformational changes of DENV polymerase during the synthesis of viral RNA that will aid the design of novel inhibitors of DENV replication.

117 **Triazolopyrimidines Are Able To Efficiently Inhibit Chikungunya Virus Replication: Synthesis and SAR.**

Maria-Jesus Perez-Perez¹, Alba Gigante¹, Leen Delang², Gilles Querat³, Dirk Jochmans², Pieter Leysen², Maria-Jose Camarasa¹, Eva-Maria Priego¹, Johan Neyts²

¹*Instituto de Quimica Medica (IQM-CSIC), Madrid, Spain*, ²*Laboratory for Virology and Experimental Chemotherapy, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium*, ³*UMR190, Emergence des Pathologies Virales, Aix-Marseille University, Marseille, France*

Chikungunya virus (CHIKV), belonging to the Alphavirus genus of the Togaviridae family, represents a health global challenge. CHIKV causes painful arthritis-like symptoms that last for months to years; in some cases, neurological complications have also been described. Its recent spread to the Caribbean islands has evidenced the potential of this virus as a real health challenge. Our laboratories have been fully involved in the identification and study of small molecules able to inhibit CHIKV replication. We have identified [1,2,3]triazolo[4,5-d]pyrimidin-7(6H)-ones as a promising family of anti-CHIKV agents, whose prototype has been coded as MADTP_314. These compounds are easily synthesized in 3 to 5 steps, and in most cases assisted by microwave irradiation. More than 100 analogues have been tested in a CPE assay. In addition, the antiviral activity of the most relevant compounds has been confirmed against several clinical isolates of CHIKV. Therefore, the present family of compounds is characterized by its simplicity in structure, synthetic accessibility and selective inhibitory activity against CHIKV replication, whose mechanism of action will also be reported in at this meeting. This research was funded by the Spanish MINECO (SAF2012-39760-C02-01) and European Union FP7 Program under SILVER grant agreement n° 260644.

Shotgun Presentations I

Chairs: **Mark Prichard, Ph.D.**

11:30 AM – 12:00 PM

ESTENSI – MEDICI

118 **Inactivation of hepatitis B virus in chronically infected cells using a CRISPR/Cas9 nickase RNA-guided endonuclease**

Jan Chemnitz¹, Madina Karimova¹, Niklas Beschorner¹, Werner Dammermann², Adam Grundhoff^{1,3}, Frank Buchholz⁴, Julian Schulze zur Wiesch^{2,3}, Joachim Hauber^{1,3}

¹*Heinrich Pette Institute – Leibniz Institute for Experimental Virology, 20251 Hamburg, Germany*, ²*Department of Medicine I, University Medical Center Eppendorf, 20246 Hamburg, Germany*, ³*German Center for Infection Research (DZIF) partner site Hamburg, Hamburg, Germany*, ⁴*Department of Medical Systems Biology, University Hospital and Medical Faculty Carl Gustav Carus, TU Dresden, 01307 Dresden, Germany*

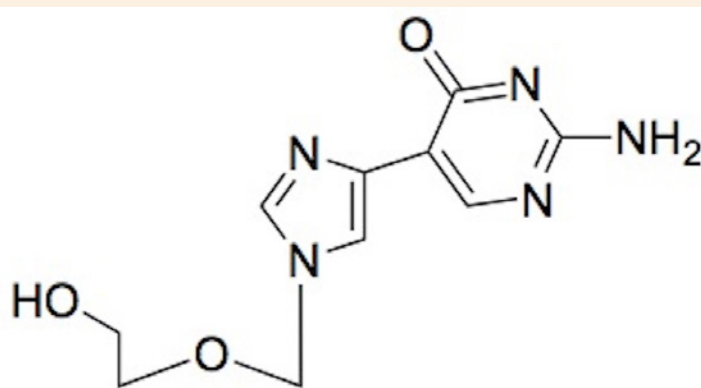
Current antiviral therapies cannot cure hepatitis B virus (HBV) infection; successful HBV eradication would require inactivation of the viral genome, which primarily persists in host cells as episomal covalently closed circular DNA (cccDNA) and, to a lesser extent, as chromosomally integrated sequences. However, novel designer enzymes, such as the CRISPR/Cas9 RNA-guided nuclease system, provide technologies for developing advanced therapy strategies that could directly attack the HBV genome. For therapeutic application in humans, such designer nucleases should recognize various HBV genotypes and cause minimal off-target effects.

Here, we identified cross-genotype conserved HBV sequences in the S and X region of the HBV genome that were targeted for specific and effective cleavage by a Cas9 nickase. This approach disrupted not only episomal cccDNA and chromosomally integrated HBV target sites in reporter cell lines, but also HBV replication in chronically infected hepatoma cell cultures. Detailed functional analyses, including next generation sequencing (NGS), demonstrated the feasibility of using the CRISPR/Cas9 nickase-system in novel therapy strategies aiming to cure HBV infection.

119 Design, Synthesis and Anti-corona Virus Activity of a Series of Acyclic Fleximer Analogues

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Replication is intrinsically involved in the lifecycle of all viruses, thus their survival relies on DNA or RNA polymerases. One of the most effective strategies in targeting this enzyme is through the use of nucleos(t)ide analogues. An area in which these analogues have been barely explored is the coronaviruses (CoVs), especially Severe Acute Respiratory Syndrome-CoV and Middle East Respiratory Syndrome-CoV. Previously it was found that a “split base” guanosine analogue (Flex-GTP) developed in our laboratory not only served as a better substrate for, but also retained full potency against, binding site mutations in guanosine fucose pyrophosphorylase due to interactions with secondary amino acid residues. Flex-GTP also served as an inhibitor of S-adenosylhomocysteine hydrolase, an adenosine-metabolizing enzyme. These observations strongly indicate that flexibility in the nucleobase scaffold can be a powerful tool for developing drugs that can bind to atypical enzymes in biologically significant conformations. Based on this information we designed and synthesized a series of novel nucleoside analogues by combining our “fleximer” base modification with various modified sugars of FDA-approved antiviral nucleoside drugs, including Acyclovir. This led to potent biological activity against a number of targets, including CoVs, with EC₅₀ values ranging from 8-51 μM. This is one of the best activity profiles for a nucleoside against coronaviruses observed to date, and has inspired a second generation of analogues, with biological testing currently underway. The results will be presented herein.



1, Flex-Acyclovir

120 Repositioning a Hepatitis C virus antiviral small molecule to treat dengue infection

Ilane Hernandez-Morales, Peggy Gelyukens, Marleen Clynhens, Rudy Strijbos, Erwin Coesemans, Benoit De Boeck, Kenneth Simmen, Frederik Pauwels, Jan Martin Berke, Koen Vandyck, Pedro Lory, Marnix Van Loock, Janssen Infectious Diseases and Vaccines, Beerse, Belgium

Dengue is the most important mosquito-transmitted viral disease with 390 million infections per year. However, there are currently no marketed antivirals or vaccines available. Dengue virus (DENV) belongs to the Flaviviridae family which also includes Hepatitis C, West Nile and Yellow Fever virus. DENV has been classified in four serotypes (DENV 1 to 4) based on its surface antigens. Upon infection; DENV non-structural proteins (NS) establish the replication complex (RC) which supports viral RNA production. In this process, NS4B anchors the RC to the endoplasmic reticulum, together with NS2A and NS4A. Although not fully understood, the role of NS4B is critical for viral replication. In addition, NS4B is highly conserved across the four serotypes, which makes it an attractive target for drug discovery.

In the present study, we identified a small molecule inhibitor of DENV replication, likely targeting NS4B. The compound was identified from a library of small molecules active against Hepatitis C virus (HCV) NS5A. In a cellular HCV replicon genotype 1b assay it showed a half-maximal effective concentration (EC₅₀) of 22 nM. For DENV, the primary screening in a DENV-2 replicon assay exhibited an EC₅₀ of 0.72 μM without toxicity in Huh7 cells (CC₅₀ >50 μM). A DENV-2/eGFP reporter full-length virus assay using Vero cells confirmed these observations. More importantly, this small molecule showed activity against all four serotypes, as determined in a tetravalent RT-qPCR assay. In contrast, the compound was not active against human Cytomegalovirus, HIV, Chikungunya, Coxsackie and Influenza viruses. *In vitro* resistance selection experiments revealed mutations in NS2B (I8V) and NS4B (L111F, I121M, I56T, T108I). In addition, using site-directed mutagenesis, T108I mutation induced a 165 fold change increase in EC₅₀ compared to wild type virus in a transient replicon assay. Interestingly, lab strains DENV3/H87 and DENV4/H241 harbor the same mutation and are insensitive to the compound.

In summary, we identified and profiled a potent tetravalent compound against DENV originating from an HCV compound library.

121 Alpha-carboxy nucleoside phosphonates as universal nucleoside triphosphate mimics

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Polymerases have a structurally highly conserved negatively charged amino acid motif that is strictly required for Mg²⁺ cation-dependent catalytic incorporation of (d)NTP nucleotides into nucleic acids. Based on these characteristics a nucleoside monophosphonate scaffold, a-carboxy nucleoside phosphonate (a-CNP), was designed that is recognized by a variety of polymerases. Kinetic, biochemical, and crystallographic studies with HIV-1 reverse transcriptase (RT) revealed that a-CNPs mimic the dNTP binding through a carboxylate oxygen, two phosphonate oxygens, and base-pairing with the template. In particular, the carboxyl oxygen of the -CNP acts as the potential equivalent of the -phosphate oxygen of dNTPs, and two oxygens of the phosphonate group of the -CNP chelate Mg²⁺ mimicking the chelation by the - and -phosphate oxygens of dNTPs. a-CNPs (i) do not require metabolic activation (phosphorylation), (ii) bind directly to the substrate-binding site, (iii) chelate one of the two active site Mg²⁺ ions, and (iv) reversibly inhibit the polymerase catalytic activity without being incorporated into nucleic acids. In addition, -CNPs were also found to selectively interact with regulatory (i.e., allosteric) Mg²⁺-dNTP-binding sites of nucleos(t)ide-metabolizing enzymes susceptible to metabolic regulation. a-CNPs represent an entirely novel and broad technological platform for the development of specific substrate active- or regulatory-site inhibitors with therapeutic potential.

122 Efficacy of a DNA-Based Live Attenuated Vaccine Against Yellow Fever Virus in a Hamster Model of Disease

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The yellow fever virus (YFV) is a mosquito-borne flavivirus that causes severe disease throughout tropical regions of Africa and South America. Although there is a safe and effective attenuated vaccine (YFV-17D, YF-VAX®), widespread vaccination campaigns are impeded by several factors including the need for a proper cold-chain, vaccine stability, need for parenteral injection and cost. To overcome these limitations, we have developed a unique and robust reverse genetics system for production of YFV-17D directly from plasmid DNA following intraperitoneal (i.p.) or transdermal (t.d.) administration. Protective efficacy of DNA-YFVax plasmid was assessed in the YF Syrian Golden hamster model and in parallel to the commercial vaccine YF-VAX®. Female hamsters (3-4 weeks of age) were vaccinated with a single i.p. dose of DNA-YFVax (25, 10, or 1 µg) 28 days prior to infection with 20 CCID₅₀ of the Jimenez hamster-adapted YFV strain. High titers of neutralizing antibodies (dose-dependent) were detected in serum 28 days after vaccination. Protection against wild-type virus challenge was observed in animals vaccinated with 25 or 10 µg of DNA-YFVax, which was comparable to the protection induced by YF-VAX®. Also, needle-free t.d. administration of DNA-YFVax resulted in protection, although somewhat less efficiently than when the vector was administered via the i.p. route. Inoculation t.d. of DNA-YFVax in mice results in the efficient induction of high titers of neutralizing antibodies, hence this route of administration will be optimized for hamsters. Significant improvement in survival, weight change, and serum alanine aminotransferase (ALT) were observed in animals vaccinated with either the 25 or 10 µg dose. Thus, we demonstrate the efficacy of DNA-YFVax in the prevention of yellow fever in a hamster model and provide data to support further development towards clinical use. [Supported by KU Leuven IOF Hefboom (IOF HB/13/010) and HHSN272201000039I Task Order A21 from the Virology Branch, NIAID, NIH]

Antiviral Targets and Mechanism of Action

Chairs: **Leen DeLang, Ph.D. and Mark Prichard, Ph.D.**

1:30 PM – 3:30 PM

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128 Evaluation of an Adenovirus Vected Filovirus Vaccine for Efficacy Against Marburg Virus Angola Aerosol Challenge of Cynomolgus Macaques

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Filoviruses are a significant global health risk with high morbidity and mortality rates, potential for person-to-person transmission, and relative stability in the environment. Furthermore, filoviruses are highly infectious, and have a low infectious dose in primates including by the aerosol route. Currently there is no vaccine available to prevent filovirus infections in humans. The disease caused by aerosolized MARV Angola in NHPs has been well characterized and shares numerous similarities with the human disease. Therefore, this model is ideal for preclinical testing of the MARV vaccine. Twelve adult male cynomolgus macaques were immunized prior to aerosol challenge with a target dose of 1000 pfu MARV Angola. Specifically, three groups of four animals were vaccinated as follows: Group 1 was vaccinated with Ad5.Mar (A) four weeks prior to challenge, Group 2 was primed with Ad26.Mar (A) eight weeks prior to challenge and boosted with Ad35.Mar (A) four weeks prior to challenge, and Group 3 which served as a negative control was primed with Ad26.empty eight weeks prior to challenge and boosted with Ad35.empty four weeks prior to challenge. Beginning on day 5 post-exposure, all four animals from the negative control group developed fever and clinical signs of disease characteristic to MARV Angola infection in cynomolgus macaques. On days 7 and 8 post-exposure, all four animals from the control group became moribund and were euthanized. All 8 vaccinated animals remained free of fever and clinical signs and survived the challenge. Negative control animals had high levels of virus in serum as detected by plaque assay; however the vaccinated animals from both vaccine groups had no live virus detected in serum. In conclusion, administration of Ad5.Mar (A) four weeks prior to challenge, or administration of Ad26.Mar (A) eight weeks prior to challenge and boost with Ad35.Mar (A) four weeks prior to challenge are fully protective against exposure to a uniformly lethal dose of airborne MARV Angola.

129 A Novel Nuclear Transport Inhibitor that Provides in vivo Protection Against Lethal Dengue Virus Infection

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Although Dengue virus (DENV) causes close to 400 million infections per year worldwide, there is currently no licensed vaccine or therapeutic. We previously showed that nuclear entry of DENV non-structural protein 5 (NS5) is central to DENV infection, with prevention of nuclear access reducing DENV virion production [1,2]. We applied a high-throughput screening approach [3] to identify compounds inhibiting interaction between NS5 and host nuclear import proteins, that we previously showed is central to NS5 nuclear localisation [1,2,4]. We identified *N*-(4-hydroxyphenyl) retinamide (4-HPR) as a lead compound, and showed it to be effective in protecting against DENV-1-4 and DENV-1-ADE infections, with 50% effective concentrations (EC₅₀s) in the low μM range [5]. 4-HPR, but not the closely related compound *N*-(4-methoxyphenyl) retinamide (4-MPR), reduced viral RNA levels and titres when applied to an established infection. 4-HPR, but not 4-MPR, was also found to up-regulate the PKR-like ER kinase (PERK) arm of the unfolded protein response (UPR). Strikingly, 4-HPR but not 4-MPR protected against infection in peripheral blood mononuclear cells, as well as in a lethal ADE mouse model. Thus, 4HPR is an antiviral modulating the UPR that is effective against DENV1-4 at concentrations achievable in the plasma in a clinical setting, and provides protection in a lethal mouse model. The results indicate that 4HPR represents an exciting possibility for future development in the fight against DENV, and imply that the UPR may be critical to the host response to infection.

[1] Pryor et al. (2007) Traffic 8, 795-807; [2] Wagstaff et al. (2012) Blochem. J. 443, 851-856; [3] Wagstaff et al. (2011) Biomol. Screen 16, 192-200; [4] Tay et al. (2013) Antivir. Res. 99, 301-306; [5] Fraser et al. (2014) J. Infect. Dis. 210, 1780-1791

130 An induced-fit binding model for nucleozin-mediated influenza A nucleoprotein (NP) aggregation

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Previously we have identified influenza A virus (IAV) nucleoprotein (NP) as a novel and effective druggable target for potential therapeutic interventions and nucleozin a potent antiviral compound with in vivo antiviral efficacies (Kao et al., 2010, Nature Biotechnology). The potential development of nucleozin-like compounds as antiviral therapeutics remains a great interest in the field as nucleozin acts on multiple stages of IVA infection by inducing massive aggregation of IAV NP and ribonucleoprotein (RNP). Despite mechanistic studies by various groups and the availability of X-ray crystal structures of NP and NP in complex with a nucleozin analogue (Gerritz et al., 2011, PNAS), the mechanism by which nucleozin induces IAV NP aggregation remains elusive. In this study, we have carried out site-directed mutagenesis on several amino acid residues in IAV NP crucial for the development of resistance to nucleozin and constructed recombinant IAV containing those mutations by reverse genetics. The three dominant resistant mutations Y289H, Y313V and Y52H and their combinations not only confer resistance to nucleozin but also abolish nucleozin-induced NP aggregation, reconciling well with the notion that the aggregation of IAV NP is the primarily mode of action of nucleozin. Examination of the two nucleozin binding sites (the “Y289 pocket” and the “Y52 pocket”) on NP (PDB files 2IQH and 3R05) has revealed that both “pockets” are sterically blocked by Y289 and Y52 respectively in its apo form (2IQH), preventing the entry of nucleozin into the binding sites. In NP’s ligand-bound form (3R05), both Y289 and Y52 flip noticeably to new positions, leading to the exposure of the ligand-binding cavities and stabilization of the bound ligand by pi-pi stacking. Once bound with nucleozin, these two induced-fit binding sites may create strong enough intermolecular forces to pull the two adjacent NP molecules tightly together, leading to the initiation of aggregation. Understanding the underlying mechanism of nucleozin-induced IAV NP aggregation will greatly facilitate structure-based design of novel IAV antivirals.

131 New structural data and molecular dynamics simulations on hepatitis C virus NS5B illuminate activation and nucleotide analog inhibition of viral RNA polymerases

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Nucleotide inhibitors of the hepatitis C virus (HCV) polymerase (NS5B) feature prominently among the direct-acting antivirals (DAA) that are currently revolutionizing HCV therapy ¹. Drugs such as sofosbuvir (Sovaldi, Gilead Sciences, Inc.) show both a very high treatment success and a high barrier to resistance. The mode of action of sofosbuvir is well understood in principle: It is an orally administered, stable nucleotide prodrug. After internalization by target cells it is metabolized into a uridine monophosphate analog. The latter is efficiently converted to the triphosphate form that can be incorporated by NS5B into the nascent viral RNA. Still, the mechanism by which NS5B actually incorporates the triphosphate metabolite of sofosbuvir remained mysterious until recently. Indeed, a ribose substitution on sofosbuvir would have been expected to interfere with a key structural element of recognition of ribonucleotides by viral RNA polymerases. Now newly published crystallographic structures of HCV NS5B in one go both solve this mystery and reveal how RNA polymerases of the *Flaviviridae* family regulate RNA synthesis initiation ². Adding these new data to the available structural and functional information ³, we performed molecular dynamics simulations that give dynamic views of HCV NS5B activation and incoming nucleotide incorporation. We will present those results, highlighting the consequences not only for closely-related viruses such as *Flaviviridae*, but also for more distant ones, including negative-sense RNA virus polymerases such as influenza virus PB1 ⁴.

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2. Appleby T C, et al. Structural basis for RNA replication by the hepatitis C virus polymerase. *Science*. 2015. 347: 771–775.
3. Cailliet-Saguy C, Lim S P, Shi P-Y, Lescar J, Bressanelli S. Polymerases of hepatitis C viruses and flaviviruses: structural and mechanistic insights and drug development. *Antiviral Res*. 2014. 105: 8–16.
4. Bressanelli S. Kickstarting a viral RNA polymerase. *Science*. 2015. 347: 715–716.

132 Biochemical Characterization of the Interaction Between ALS-8112 Triphosphate and the RNA-Dependent RNA Polymerase of Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections in infants, and yet no vaccines or effective therapeutics are currently available. Here, we present the mechanism of action of ALS-8176, a first-in-class nucleoside analog prodrug that is currently under evaluation in the clinic for the treatment of RSV infection in hospitalized infants. The parent cytidine analog, ALS-8112, inhibits all tested strains of RSV in vitro, but it is selective for non-segmented negative-strand RNA viruses. The mechanism of action of ALS-8112, which involves the inhibition of the viral polymerase and is mediated by its 5'-triphosphate form (ALS-8112-TP), was supported by two orthogonal methods. Firstly, ALS-8112 selected for resistance-associated mutations within the region of the L gene of RSV encoding the RNA-dependent RNA polymerase. Secondly, ALS-8112-TP was efficiently incorporated by the RSV polymerase and caused complete and immediate chain termination of RNA synthesis. ALS-8112-TP did not interact with viral or human RNA and DNA polymerases unrelated to RSV. The potency and selectivity of ALS-8112-TP could be rationally explained by a comprehensive structure-activity relationship analysis of the modifications within the sugar moiety. Overall, these preclinical results demonstrate that ALS-8112-TP is a very potent and selective chain terminator of RSV polymerase. The in vitro antiviral profile of ALS-8112 and its triphosphate counterpart support the recent human challenge study where ALS-8176 given orally was efficacious against RSV infection in adult volunteers.

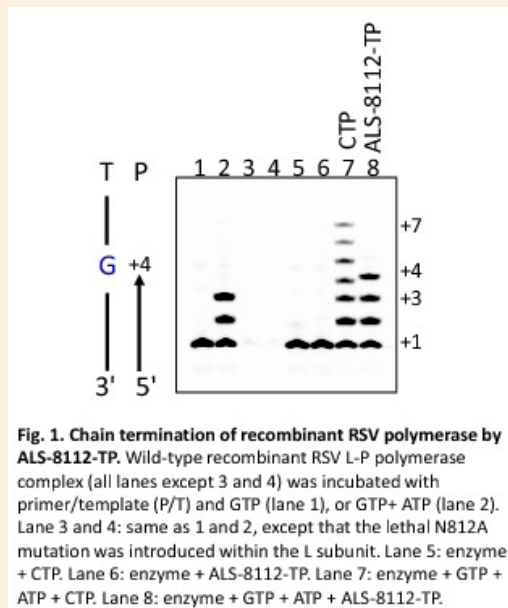


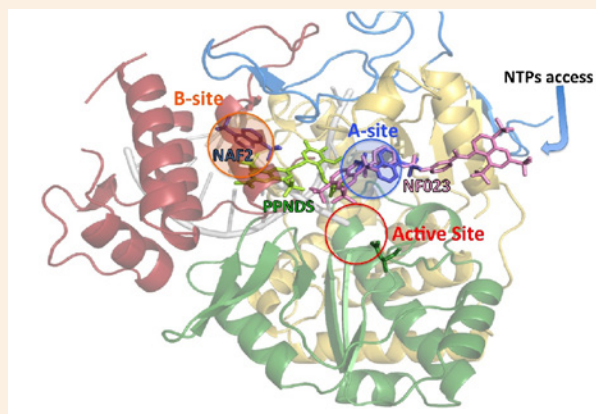
Fig. 1. Chain termination of recombinant RSV polymerase by ALS-8112-TP. Wild-type recombinant RSV L-P polymerase complex (all lanes except 3 and 4) was incubated with primer/template (P/T) and GTP (lane 1), or GTP+ ATP (lane 2). Lane 3 and 4: same as 1 and 2, except that the lethal N812A mutation was introduced within the L subunit. Lane 5: enzyme + CTP. Lane 6: enzyme + ALS-8112-TP. Lane 7: enzyme + GTP + ATP + CTP. Lane 8: enzyme + GTP + ATP + ALS-8112-TP.

133 Combating Norovirus-dependent gastroenteritis through RdRp inhibitors

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Noroviruses (NV) are members of the Calciviridae family of positive sense RNA viruses, which cause rapid onset diarrhea and vomiting. Currently, NV infection causes over 200,000 deaths/year. Nevertheless, no effective vaccines/antivirals are yet available to treat NV infection. Since the activity of RNA-dependent RNA polymerase (RdRp) plays a key role in genome replication, this enzyme is considered a promising target for antiviral drug development. In this context, we identified suramin and NF023 as NV RdRp inhibitors that, however, are hampered by pharmacokinetics problems. To overcome such problem, we followed two ways: on one hand we produced different suramin-loaded liposome formulations, showing that suramin, when delivered through liposomes, can effectively inhibit murine NV replication in RAW264.7 macrophages. On the other hand, we analyzed the potential inhibitory role of naphthalene-sulfonate (i.e. NAF2) and of the related molecule PPNDS. The crystal structures of NV RdRp in complex with NAF2 or PPNDS revealed a new binding site that differs from that characterized for NF023/suramin (Fig.1). To further map the new potential inhibitory site, we focused on structurally related molecules that were synthesized following structure-driven information. The crystal structures of NV RdRps in complex with one of such compounds provided new insight on the interactions that a small fragment can establish with NV RdRps, and establishing a platform for structure-based optimization of potency, selectivity and drugability.



134 Evaluation of the histone deacetylase inhibitor ST7612AA1 as an HIV-1 latency reactivation agent

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Antiretroviral therapy (ART) is unable to cure HIV infection. The ability of HIV to establish a subset of latent infected CD4+ T cells, which remain undetectable to the immune system, becomes a major roadblock to achieve viral eradication. Histone deacetylase inhibitors (HDACi) have been shown to potently induce the reactivation of latent HIV. Here, we show that a new thiol-based HDACi, the thioacetate-w(g-lactam carboxamide) derivative ST7612AA1, is a potent inducer of HIV reactivation.

We evaluated HIV reactivation activity ST7612AA1 compared to Panobinostat (PNB), Romidepsin (RMD) and Vorinostat (VOR) in an in vitro model of HIV-1 latently infected lymphoid Jurkat (J-Lat) cells, cultured for 48h in the presence of HDACi, or phorbol 12-myristate 13-acetate (PMA) and ionomycin as controls. ST7612AA1 potently induced HIV-1 reactivation of J-Lat cells at submicromolar concentrations. HIV reactivation occurred in the presence of combinations of antiretrovirals and, the activity of ART was not affected by ST7612AA1 in acutely infected CD4+ lymphocytes. The ability of ST7612AA1 to induce cell proliferation and/or cell activation was evaluated in CD4+ T cells, cultured for 48h in the presence of the corresponding HDACi supplemented with interleukin-2. Resting CD4+ T cells and CD4+ T cells supplemented with PMA+ionomycin and anti-CD3/anti-CD28 were used as controls. Cell proliferation, as measured by ki67 intracellular marker, showed that none of HDACi tested induced T cell proliferation at subtoxic concentrations. Similarly, cell activation markers CD25, CD69, CD38 and HLA-DR were not affected by ST7612AA1, PNB, RMD or VOR.

In conclusion, our results indicate that ST7612AA1 is a potent activator of latent HIV and that the reactivation activity of ST7612AA1 is exerted without activation or proliferation of CD4+ T cells. ST7612AA1 is a suitable candidate for further studies of HIV reactivation strategies and potential new therapies to eradicate the viral reservoirs.

135 Quinoxaline-6-carboxamides inhibit HBV infection in vitro

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Currently, more than 350 million people suffer from chronic HBV infection. Chronic hepatitis B frequently progresses to liver cirrhosis and hepatocellular carcinoma, a leading cause of cancer-related morbidity and mortality worldwide.

This study is focused on the discovery and characterization of small molecules that reduce or eliminate HBV cccDNA from the nuclei of infected cells. Drug candidates with such mechanism of action, in contrast to the currently available HBV drugs, would have a potential to eradicate HBV and cure chronically-infected HBV patients.

A robust HBV *in vitro*-infection model has been developed (Seeger and Sohn, 2014). A human hepatoma cell line HepG2, stably expressing the sodium taurocholate cotransporting polypeptide (NTCP), has been constructed and characterized. An efficient HBV replication in the infected cells was confirmed with cell ELISA for several viral intracellular antigens (including HBsAg- Large, HBsAg-Middle and HBcAg), as well as with immunofluorescence and immunohistochemistry.

The HTS was automated and run on the Biomek robotic workstation. The HepG2/NTCP cells in the 96-well plates were infected with HBV, treated with test compounds at 10 μ M for 7 days, and HBV replication inhibition was measured using ELISA for the secreted HBeAg as readout. Compound cytotoxicity was determined in parallel, using cells in same 96-well plates.

The antiviral activity and cytotoxicity of the identified hits was further evaluated at multiple concentrations, and the EC50 and CC50 values were determined. As a result, at least five distinct chemistry series of HBV inhibitors were identified, with the EC50 values ranging from 0.1 +/- 0.03 μ M to < 10 μ M, and CC50 values >30 μ M (the highest concentration tested). The HBV inhibition was also confirmed by immunofluorescence staining for the intracellular HBcAg. The mechanism of molecular action studies for the most promising inhibitor series are in progress. The results of these studies, including time of drug addition experiments, compound effects on HBV core DNA and HBV cccDNA in cell cultures using the real-time quantitative PCR technique, will be presented. In conclusion, further pre-clinical studies of these newly discovered HBV inhibitors are warranted.

136 **A novel class of chikungunya virus inhibitors targets the enzymatic activity of the viral capping enzyme nsP1.**

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The last decade, chikungunya virus (CHIKV) re-emerged in many parts of Africa and Asia, and recently also for the first time in Central and South America. The global re-emergence of this virus and its high morbidity rate emphasize the need for potent antivirals for treatment. We recently identified MADTP_314, a [1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one, as a potent inhibitor of CHIKV replication with no toxicity to the host cells. The antiviral activity of this molecule was confirmed against several clinical isolates of CHIKV, including the Caribbean St-Martin strain. To identify the viral protein(s) involved in the mechanism of action of this molecule, drug-resistant CHIKV variants were isolated. In all independently-selected drug-resistant isolates, a single mutation was identified in the nsP1 coding sequence resulting in a P34S amino acid substitution. Reverse-engineering corroborated the link between this mutation and the compound-resistant phenotype. nsP1 is the central enzyme for the viral mRNA capping and interestingly, the proline at position 34 is located near the conserved histidine (H38) in a region involved in methyltransferase/guanylyltransferase functions. Enzymatic assays using the nsP1 of Venezuelan equine encephalitis virus (VEEV) showed that MADTP_314 did not, or poorly, interfere with the N7 methylation of GTP performed by nsP1 but rather inhibited the covalent binding of N7-GMP on H38 of nsP1. Interestingly, analogues of MADTP_314 with more potent anti-CHIKV activity did not only inhibit the guanylyltransferase activity but also the methyltransferase activity of nsP1. Moreover, the corresponding resistance mutation on VEEV nsP1 (D34S) abrogated the inhibitory effect of MADTP compounds on nsP1 capping functions. In conclusion, a novel class of CHIKV inhibitors targeting the viral capping machinery was discovered.

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137 **A novel class of host-directed antivirals with broad spectrum activity against respiratory and systemic RNA viruses.**

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We have identified a broad spectrum antiviral candidate with low nM potency in multiple respiratory and emerging pathogens that functions through a novel host mediated target. Broad-spectrum antivirals that retain potency in the presence of rapidly evolving viruses are in demand and host-directed antivirals remain an attractive therapeutic strategy. We previously reported the identification of a novel class of chromenone drug candidates that modulate innate immunity along the RLR-IRF3 axis. Importantly, our compounds activate innate immune signaling downstream of numerous viral countermeasures and are a unique addition to conventional antiviral compounds in development or on the market. Through SAR, we have achieved broad spectrum in vitro activity against diverse RNA viruses including the respiratory pathogens, influenza, RSV, and hCoV with EC50s in the low nM range. While our development path is focused on broad respiratory indications, we have demonstrated potent in vitro activity against systemic and emerging viruses including dengue and ebola. Administration of lead compounds provides significant therapeutic benefit in murine models of respiratory infection, including influenza and RSV. Prophylactic treatment reduces titers over 2 logs and more than one log in a delayed treatment model, translating to significant decreases in morbidity and mortality. These analogs demonstrate high permeability and sufficient metabolic stability to provide oral bioavailability compatible with twice daily oral dosing. Finally, lead molecules exhibit a generous therapeutic index, show no off-target receptor activation and no genotoxic or cardiotoxic potential. In summary, our lead compounds proposed for development are non-direct acting antivirals that potentiate a cell autonomous effector response active against diverse RNA viruses, are less likely to elicit emergence of resistant viral variants, and have potential to be therapeutics for viral infections of undiagnosed etiology.

138 **T-705 and ribavirin induce lethal mutagenesis of influenza virus**

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The nucleobase analogue T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide; favipiravir) is a unique antiviral compound with broad and selective anti-RNA virus activity and a high barrier for resistance. Two hypotheses have been proposed for its mechanism of action (MOA) as anti-influenza virus agent: lethal virus mutagenesis and non-obligate chain termination.

When we passaged influenza virus in suboptimal concentrations of T-705 (3.2-20 µM), we observed that viral infectivity rapidly declined. After 14 passages, deep-sequencing of the entire viral genome revealed that T-705 increased the mutation rate by 4- to 16-fold. The majority (67%) of the mutations were G-to-A or C-to-U transitions, which is consistent with T-705 being a pseudobase mimicking both guanine and adenine due to its rotating carboxamide. These observations concur with a recent report (Baranovich et al., J. Virol., 2013) that T-705 acts as a lethal virus mutagen. Importantly, while these authors detected only a few missense mutations in the viral neuraminidase gene, we identified on average 27 mutations, of which one-third were missense, in the viral ribonucleoprotein (vRNP) components, i.e. PB1, PB2, PA and NP.

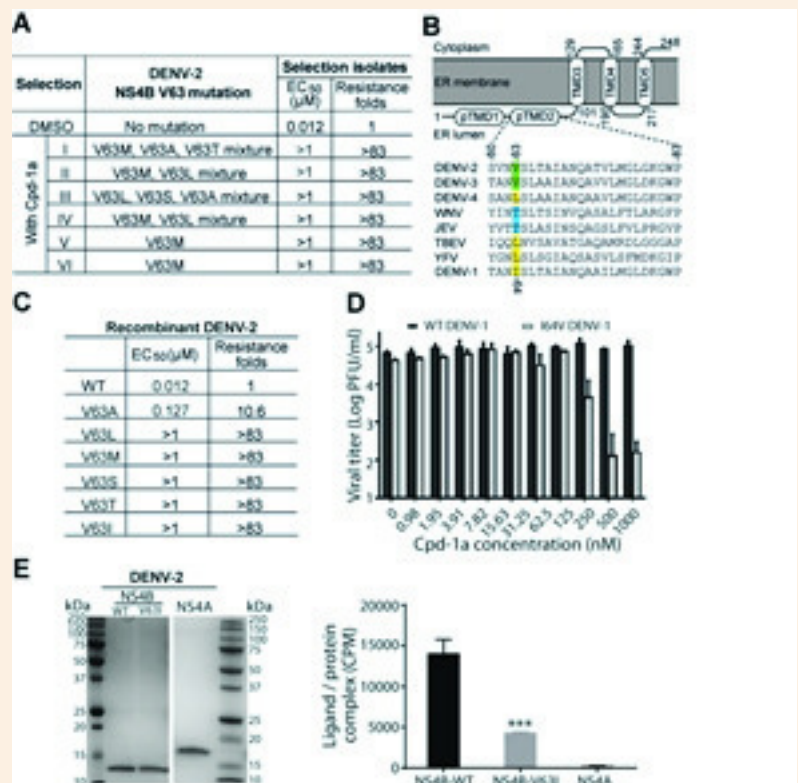
The rotating carboxamide and dual MOA of T-705 are reminiscent of ribavirin, another antiviral to which different MOAs have been attributed. Similar passaging experiments with ribavirin resulted in a 2- to 15-fold increased mutation rate, and 86% were G-to-A or C-to-U transitions. On average, 16 mutations were located in the vRNP proteins, of which half were missense.

The accumulation of mutations under T-705 or ribavirin resulted in a significant reduction in viral replication fitness, i.e. by a factor 1800 (T-705) and 120 (ribavirin) in the 14th virus passage, as determined by titration in cell culture and RT-qPCR.

139 **Discovery of dengue virus NS4B inhibitors**

QING-YIN WANG^{1,1}, HONGPING DONG^{1,1}, BIN ZOU², PAUL SMITH³, PEI-YONG SHI⁴
^{1,1} SINGAPORE, Singapore, ^{2,2} SINGAPORE, Singapore, ^{3,3} SINGAPORE, Singapore, ^{4,4} Corresponding, SINGAPORE, Singapore

The four serotypes of dengue virus (DENV-1 to -4) represent the most prevalent mosquito-borne viral pathogen in humans. No clinically approved vaccine or antiviral is currently available for DENV. Here we report a spiroprazolopyridone compound that potently inhibits DENV both in vitro and in vivo. The inhibitor was identified through screening a 1.8-million compound library using a DENV-2 replicon assay. The compound selectively inhibits DENV-2 and -3 (EC₅₀ 10-80 nM), but not DENV-1 and 4 (EC₅₀ >20 µM). Resistance analysis showed a mutation at amino acid 63 of DENV-2 NS4B (a non-enzymatic transmembrane protein from viral replication complex) could confer resistance to compound inhibition. Genetic studies demonstrate that variations at amino acid 63 of NS4B protein are responsible for the selective inhibition of DENV-2 and -3. Medicinal chemistry improved the physicochemical properties of the initial "hit", leading to compound-14a that has good in vivo pharmacokinetics. Treatment of DENV-2-infected AG129 mice with compound-14a suppressed viremia, even when the treatment started two days after viral infection. The results have proved the concept that inhibitors of NS4B could potentially be developed for clinical treatment of DENV infection. Compound-14a represents a preclinical candidate for potential treatment of DENV-2 and -3 infected patients.



Poster Session II

4:00 PM – 6:00 PM

FERNANDES

141 Studies on anti-HIV activity, cytotoxicity of wrightia tinctoria R.Br. Leaf

P Selvam¹, Akhil C. Banerjee²

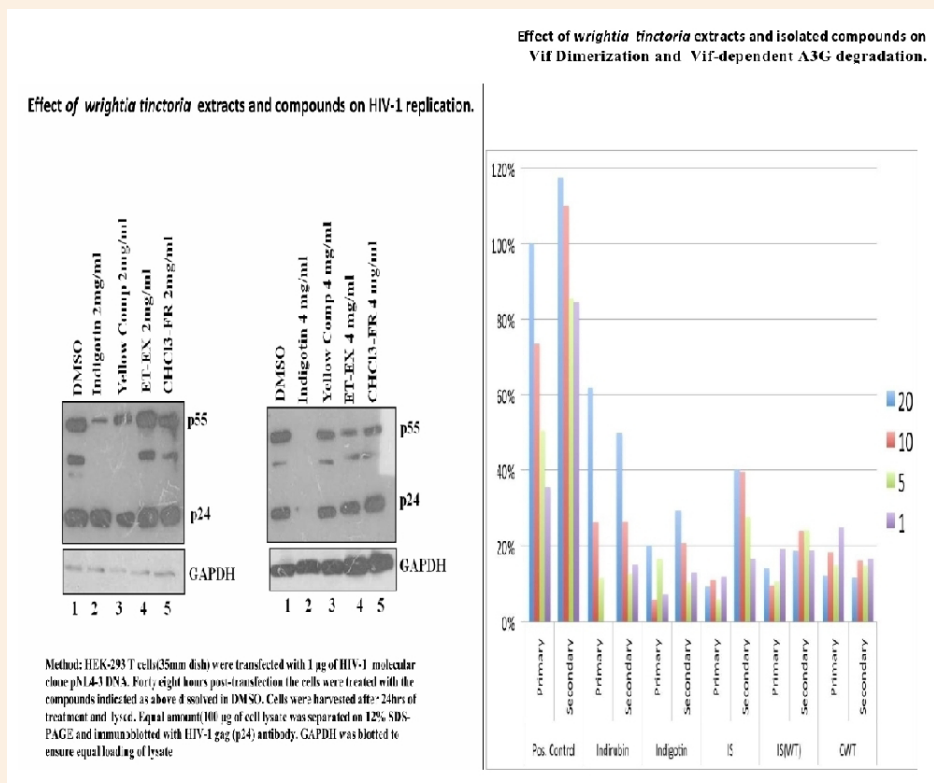
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BACKGROUND: *Wrightia tinctoria*/i R.Br. (Apocynaceae) used in Indian system of medicine as immunomodulator. *Wrightia tinctoria*/i (WT) leaf extracts documented for inhibition of HIV integrase enzymatic activity (Selvam et al./i 2010). Present work is to investigation of anti-HIV activity, cytotoxicity of extracts and isolated compounds of WT leaf and also studied for inhibition of Vif Dimerization and Vif-dependent A3G degradation to understand further molecular mechanism.

METHODS: Different extracts prepared from WT Leaf powder and extracts were subjected to column chromatography for the isolation of active compounds. Extracts and isolated compounds were investigated for anti-HIV activity against HIV -1 replication in HEK 293 cells by P-24 antigen assay, Cytotoxicity also investigated in WT extracts and isolated also investigated for Vif Dimerization and Vif-dependent A3G degradation.

RESULTS: Isolated compound indigotin (dimer of isatin) inhibits the HIV-1 P 24 antigen gene expression in HEK 293 cells (Fig 1). Isatin (2,3-dioxindole) and Indirubin (dimer of isatin) is identified as novel lead molecules for inhibition of Vif Dimerization and Vif-dependent A3G degradation (Fig 1). Indole derivatives isatin, indirubin and indigotin are the novel classes for lead molecules for designing potential anti-HIV agents with novel mechanism.

P.Selvam,et al./i Studies of HIV-1 Integrase Inhibitory Activity of *Wrightia tinctoria*/i Antiviral Res, 8,2010.



142 Evasion of innate immunity mediated by orf virus protein OV20.0

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Orf virus (ORFV) *OV20.0L* is an ortholog of vaccinia virus (VACV) gene *E3L*. The function of VACV E3 protein as a virulence factor is well studied, but *OV20.0* has received less attention. Here we show that like VACV *E3L*, *OV20.0L* encodes two proteins, a full-length protein and a shorter form (sh20). These isoforms differed in cellular localisation, with full-length *OV20.0* and sh20 found throughout the cell and predominantly in the cytoplasm, respectively. Similar to the full length isoform, sh20 is able to bind dsRNA and PKR, inactivate PKR and thus act as an antagonist of the interferon response in vitro. In spite of this apparent conservation of function in vitro, a recombinant ORFV that could only express the sh20 isoform was attenuated in a mouse model, suggesting that the functions of the isoforms are not simply redundant.

143 The antiviral effect of favipiravir (T-705) on Coxsackievirus B3 replication is modulated by the nature of the amino acid residue at the highly conserved position 159 of the RNA-dependent RNA polymerase

Rana Abdelnabi¹, Pieter Leyssen¹, Bruno Canard², Johan Neyts¹, Leen Delang¹

¹Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, ²AFMB, Aix-Marseille Université, Marseille, France

Favipiravir (T-705) is a broad-spectrum antiviral agent that has been approved in Japan for the treatment of influenza. T-705 is metabolized intracellularly to its ribofuranosyl 5'-triphosphate form that competitively inhibits the incorporation of ATP and GTP by the viral RNA-dependent RNA polymerase (RdRp). T-705 also inhibits the replication of chikungunya virus (CHIKV) (Delang *et al*, J Antimicrob Chemother 2014). Low-level T-705-resistant CHIKV variants were selected. A K291R mutation in the F1 motif of the CHIKV RdRp was linked to this observed resistance. Interestingly, K291 is a highly conserved residue in the RdRps of +ssRNA viruses. Coxsackievirus B3 (CVB3) is 4-fold less sensitive to T-705 than CHIKV. Introduction of a K-to-R mutation at the corresponding position (K159R) in the F1 motif of the CVB3 RdRp results in a non-viable variant that requires a compensatory mutation [A239G] to replicate. Interestingly, the A239G mutant was previously reported as a low fidelity RdRp variant (Gnädig *et al*, Proc Natl Acad Sci USA 2012). To explore the importance of the residue at position 159, a mutation analysis was performed: only 3 more variants were viable (K159M-A239G, K159T and K159V). The reverse-engineered A239G, K159R-A239G and K159M-A239G variants were more susceptible than wild-type (WT) to the antiviral effect of T-705. In contrast, the K159V variant showed low-level resistance to T-705 (1.7-fold, $p < 0.0001$), whereas the K159T variant behaved similar to WT. The antiviral activity of T-705 against WT and the A239G and K159V variants could be completely reversed by the addition of exogenous guanosine. However, the K159R-A239G and K159M-A239G variants were markedly less sensitive to this reversion. Kinetic and fidelity studies with these RdRp variants will be reported. This should yield novel insights into the mechanism by which T-705 exhibits broad-spectrum antiviral activity and also into the role of the conserved lysine in the F1 motif of the RdRp of +ssRNA viruses.

L.D. is funded by (FWO). This project is supported by EuFP7 project, SILVER, a BELSPO grant and KULeuven GOA

145 Development and Characterization of Surface Modified Chitosan Nanoparticles for Selective Targeting of Lamivudine to Hepatocyte

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Hepatitis B is an infection of the liver caused by the hepatitis B virus (HBV). It is a major cause of infectious liver disease throughout the world. Viral hepatitis resides primarily in the liver; hence drug targeting with ligand anchored moiety can be an effective strategy in management of this disease. Lamivudine a “nucleoside analogue” is commonly used in treatment of Hepatitis B and effectively inhibit viral replication. However it shows extra-hepatic toxicity.

In the light of above, it was envisaged that the use of receptor-mediated endocytosis may permit the realization of the potential of drug targeting that reduces the side effects. This necessitates developing surface modified chitosan nanoparticles for hepatocyte selective targeting via conjugation of a ligand (glycyrrhizin).

The chitosan nanoparticles were prepared by Low Molecular Weight Chitosan (LMWC) by Ionotropic gelation method and ligand was anchored. The nanoparticles were then characterized *in-vitro* for their shape, size, drug entrapment, in-vitro drug release and stability. The *in-vivo* study comprised of biodistribution studies in various organs and fluorescence microscopy was performed, hematological and histological examinations were done.

Finally it could be concluded that encapsulation of lamivudine in glycyrrhizin coupled LMWC nanoparticles enhances the residence time. Further bioavailability of the drug in liver is increased which could be utilized in reducing the dosing frequency as well as the dose. This could help in the reduction of dose related toxicity associated with this antiviral drug. Ligand mediated bio-deposition and cellular interaction of LMWC nanoparticles especially at the site would be a focal paradigm for the upcoming research in the field of antiviral drug delivery.

146 Lipid based Nanoparticulate system for effective vaccine delivery

Gomed Agarwal¹, Saurabh Bhargava², Vishal Bhargava¹

¹KRV Hospitals Pvt. Ltd., Kanpur, India, ²Manav Bharti University, Kanpur, India

The search for innovative ways of vaccination has intensified recently with declining vaccine coverage and growing public concern about new virulent disease outbreaks. Immunization is a prophylactic approach through which the body is shielded from any incoming pathogenic invasion.

The work envisaged here concerns exploring potential of Solid Lipid Nanoparticles(SLN) in efficient protein delivery through surface modifications, which will in turn; enhance loading efficiency and cellular uptake of SLN using subcutaneous route.

The SLN were prepared by Solvent Injection Method. SLN were optimized for various parameters such as lipid concentration, surfactant concentration, stirring time and stirring speed. By considering particle size, polydispersity index (PI) and entrapment efficiency. The characterization parameters included Transmission & Scanning Electron Microscopy, X-Ray Diffraction Analysis, *In-vitro* release, Kinetics of uptake by flow cytometer, Evaluation of cell apoptosis, T-cell proliferative response assay, TH1/TH2 cytokine profile and Internalization studies by spectral bioimaging. The *in-vivo* study comprised of fluorescence studies and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

The particulate system is better carrier system for immunization because of less diffusivity and restricted movement. SLNs themselves act as signal for the phagocytic cells. Surface modified SLNs can entrap greater amount of antigen, provide its sustained release and rapidly internalized by the antigen presenting cells. *In-vitro* T cell proliferation and induction of TH1 type of immune response clearly marks the potential of this novel carrier system. Fluorescence uptake studies showed better uptake of surface modified SLNs. Higher and more sustained antibody titer obtained with surface modified SLNs suggests their better immunological potential. Thus, subcutaneous immunization could be an efficient alternative approach for vaccination against hepatitis.

The formulations developed in this study can be further explored for the incorporation and delivery of other proteins and peptides and should subsequently be subjected to pilot plant scale-up as well as clinical trial to establish their potential for subcutaneous immunization against hepatitis B.

147 Antibody coated Liposomes for Transmucosal vaccination

Manee Agarwal¹, S Bhargava²

¹ICFAI University, Kanpur, India, ²Manav Bharti University, Kanpur, India

The critical role of vaccine delivery system in “rational vaccine design” has been widely recognized. The object of the study was to investigate the feasibility of a controlled release hepatitis B vaccine by improving the stability & biocompatibility of the system by delivering the antigen on a mucosal surface. Thus research work was envisaged involving development of antibody coated liposome for transmucosal immunization against hepatitis B which may offer increased uptake of nanoliposome through transmucosal surface of nasal route and sustained release of hepatitis B surface antigen and evoked relatively high IgA titre in mucosal surface. Liposomes were prepared by a lipid cast film method & then IgG antibody was cross linked on the surface. Coated liposomes were characterized *in-vitro* for their shape, size, percent antigen entrapment and stability. Fluorescence microscopy was performed to confirm the deposition pattern of the formulation in the respiratory tract. The *in-vivo* part of the study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. Liposomes formed were multilamellar & stable. Observation of fluorescence images of nasal mucosa, lungs and spleen, revealed that these antibody coated liposome, were significantly taken up by mice respiratory mucosal surface, which made them promising carriers for mucosal vaccination. Considerable immune responses were produced by the developed system that may be due to the induction of MALT as well as contribution of the peripheral airways. The higher immunity induced by ACL-HBsAg may be attributed to its cationic nature, antibody coating and subsequent mucoadhesive property. Thus mucosal immunization with lipid vesicle through nasal administration may be effective in prophylaxis of diseases transmitted through mucosal routes as well as systemic infections e.g. Hepatitis B and others. The strategy can be made more appropriate by determination of paracellular transport, nasal mucociliary clearance, mucosal toxicity assessment etc. The approach can be expanded in many areas and may be acceptable clinically only after obtaining reproducible data in larger animal populations and primates.

148 SMADs, IRFs and antiviral innate immunity: defining the intersection of BMP and IFN signalling at the genomic level

Kinda Al-Hourani, Lucy Eddowes, Hal Drakesmith

MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom

Previous work in our lab has shown that bone morphogenetic proteins (BMPs) can enhance interferon (IFN) activity, potentially improving antiviral immunity. However, the mechanistic basis for the boosting of IFN by BMPs is unclear and needs elucidation before the antiviral effects we have found can be translated into therapies.

Our experimental observations strongly hint towards intersection of IFN and BMP pathways at the level of transcriptional regulation. SMAD transcription factors transduce BMP signalling, whereas IRFs regulate IFN. Crystal structures show topological homology between SMAD and IRF proteins, and SMAD-IRF heterodimers have been observed and functionally regulate gene expression. To address whether a manifestation of this interaction is detectable at the genomic level, we are embarking upon a series of bioinformatic analyses predicated upon three questions:

- 1) Genome-wide, do SMADs bind DNA near to known antiviral genes?
- 2) Is there similarity in the genomic binding profiles of SMAD and IRF transcription factors?
- 3) Is there evidence of SMAD and IRF co-binding at antiviral loci?

We have initiated our study by analysing the most appropriate publicly available ChIP-seq datasets. These are derived from K562 cells treated with IFN and BMP4, and show genome-wide binding profiles of IRF1 and SMAD1 respectively.

Gene set enrichment analysis of the BMP4-SMAD1 dataset reveals enrichment of BMP molecular functions, as expected, but also enrichment of IFN and antiviral responses across all ontology databases interrogated. Multiple large and directly overlapping SMAD1 and IRF1-peaks are present in genes encoding well-validated antiviral effectors. The read distributions from each dataset indicate enrichment for SMAD1-associated reads about the centre of peaks from the IFN-IRF1 dataset and vice versa. Analysis of transcription factor binding motif incidence demonstrates enrichment for IRF-associated motifs proximal to peaks identified in the BMP4-SMAD1 dataset.

Our experimental and bioinformatic studies thus far point to genome-level cross-talk between the BMP and IFN pathways, an interaction that may have implications for the development of therapies for diverse infections.

149 Triple mode of anti-viral action of Verapamil against influenza virus replication

Intakhab Alam¹, Pumaree Kanrai¹, Julia Dzieciolowski¹, Ahmed Mostafa¹, Mueller Christin¹, John Ziebuhr¹, Ursula Dietrich², Stephan Pleschka¹

¹*Institute of Medical Virology, University of Giessen, Giessen, Germany,* ²*Georg-Speyer-Haus, Frankfurt, Germany*

Repetitive emergences of influenza viruses (IV) that are capable to infect humans with a high case fatality and that show a pandemic potential (H5N1-1997/2003, H7N9-2013) raise a strong concern. Preventive vaccination against such unprecedented foes is not in sight and the approved anti-virals are not only limited in numbers, but also prone to loose their effectiveness due to viral resistance. Therefore strategies targeting cellular factors or mechanisms, which are essential for IV propagation, have come into focus over the last decade. Here, we further analysed the anti-viral activity of the calcium channel inhibitor Verapamil against IV propagation. We demonstrate that Verapamil exerts three distinguishable modes of actions against IV propagation.

150 Suramin Inhibits Chikungunya Virus Replication Through Multiple Mechanisms

Irina Albuлесcu, Marcella van Hoolwerff, Ali Tas, Florine Scholte, Eric Snijder, Martijn van Hemert

Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes severe persistent arthritis and has affected the lives of millions since its re-emergence in 2005, including an estimated 1 million infections over the past year during the first CHIKV outbreak in the Americas. We have previously developed an in vitro assay for CHIKV RNA synthesis as a tool for mechanistic studies on replication/transcription complexes (RTCs) and mode of action studies on compounds with anti-CHIKV activity. The anti-parasitic drug suramin was previously shown – after in silico docking studies- to inhibit the in vitro activity of the norovirus RNA polymerase (Mastrangelo et al., J. Mol. Biol. 419: 198-210). We found that suramin also inhibits CHIKV RNA synthesis in our in vitro assay. Also in cell culture suramin inhibited CHIKV replication with an EC50 of ~85 µM (CC50 >500 µM). The compound is active against various CHIKV isolates, and also inhibits Sindbis virus and Semliki Forest virus. In the in vitro assay suramin directly inhibits RNA synthesis with an IC50 of ~5 µM. The higher EC50 was probably due to inefficient uptake of the compound during cell-based assays. In vitro experiments suggested that suramin does not affect RTCs already engaged in RNA synthesis, but rather interferes with (re)initiation. Time-of-addition studies and experiments with transfected CHIKV (replicon) RNA suggested that suramin also interferes with an early step in infection, possibly binding and/or entry, as was also observed for other viruses. To obtain more information on the mode of action, we are currently selecting suramin-resistant viruses and are performing reverse genetics studies –based on published mutations in suramin-resistant norovirus RdRps- to assess the role of specific CHIKV nsP4 residues in sensitivity to suramin. Favipiravir- or ribavirin-resistant CHIKV (nsP4) mutants did not exhibit cross-resistance to suramin, suggesting different modes of action. Suramin is a useful tool for in vitro studies to gain mechanistic insight into CHIKV RNA synthesis. Its approved status makes it worthwhile to explore the use of suramin to treat and/or prevent CHIKV infections. Supported by EU-FP7 grant EUVIRNA (#264286).

151 Uptake and Metabolism of the HPV Antiviral, Octadecyloxyethyl benzyl 9-[2-(phosphonomethoxy)ethyl]guanine, in HFF and HEK-293 Cells

Kathy A. Aldern¹, James R. Beadle¹, Nadejda Valiaeva¹, Karl Y. Hostetler^{1,2}

¹*University of California, San Diego, La Jolla, USA,* ²*Hera Therapeutics Inc, Del Mar, USA*

Nearly all cervical cancers are caused by the high-risk genotypes 16 and 18 of the human papillomavirus (HPV). Although vaccines are in use, there are currently no FDA-approved treatments for persons already infected with the virus and preventive vaccines are not effective for this population. We have shown that octadecyloxyethyl benzyl 9-[2-(phosphonomethoxy)-ethyl]guanine (ODE-bn-PMEG) selectively inhibits HPV 11 DNA replication. To evaluate its cellular metabolism, we prepared ODE-bn-[8-¹⁴C]PMEG in collaboration with Moravek Biochemicals (Brea, CA) and studied its uptake and intracellular metabolic conversions in human foreskin fibroblast (HFF) and human embryonic kidney (HEK-293) cells. When either cell type was exposed to 1 µM of ODE-bn-[8-¹⁴C]PMEG for up to 48 hr, rapid cellular uptake was noted. Further evaluation by Partisil SAX HPLC allowed for measurement of the cellular levels of PMEG, PMEGp, and PMEGpp, the active metabolite, over 48 hr. ODE-bn-[8-¹⁴C]PMEG was slowly converted to PMEG followed by rapid metabolism to PMEGp and PMEGpp, the principal product. To assess the intracellular persistence of PMEGpp, HEK-293 and HFF cells were exposed to ODE-bn-[8-¹⁴C]PMEG for 24 or 48 hr, respectively, followed by removal of the media and replacement with drug-free media. The metabolite levels were analyzed at intervals up to 14 days. Remarkably, the PMEG diphosphate levels increased for 10 to 11 days and then declined gradually. These studies suggest that infrequent topical dosing with ODE-bn-PMEG could fill an important unmet need as a treatment for cervical and other ano-genital infections with high-risk HPV genotypes.

152 Uptake and Metabolism of the HPV Antiviral, Octadecyloxyethyl benzyl [8-¹⁴C] 9-[2-(phosphonomethoxy)ethyl]guanine, in HFF and HEK-293 Cells

Kathy A. Aldern, James R. Beadle, Nadejda Valiaeva, Karl Y. Hostetler
Univ California San Diego, La Jolla, USA

Seventy percent of cervical cancers are caused by the high-risk genotypes 16 and 18 of the human papillomavirus (HPV). Although vaccines are in use, there are currently no FDA-approved treatments for persons already infected with the virus. The preventive vaccines are not effective for this population. We have shown that octadecyloxyethyl benzyl 9-[2-(phosphonomethoxy)ethyl] guanine (ODE-Bn-PMEG) selectively inhibits DNA amplification of HPV high risk types 16 and 18. We prepared ODE-Bn-[8-¹⁴C]PMEG in collaboration with Moravek Biochemicals (Brea, CA) and studied its uptake and intracellular metabolic conversions in human foreskin fibroblast (HFF) and human embryonic kidney (HEK-293) cells. When either cell type was exposed to 1 μM of ODE-Bn-[8-¹⁴C]PMEG for up to 48 hr, rapid cellular uptake was noted. Further evaluation by Partisil SAX HPLC allowed for measurement of the cellular levels of PMEG, PMEGp, and PMEGpp, the active metabolite, over 48 hr. ODE-Bn-[8-¹⁴C]PMEG was slowly converted to PMEG followed by rapid metabolism to PMEGpp, the principal product. To assess the intracellular persistence of PMEGpp, HEK-293 and HFF cells were exposed to ODE-Bn-[8-¹⁴C]PMEG for 24 or 48 hr, respectively, followed by removal of the media and replacement with drug-free media. The metabolite levels were analyzed at intervals up to 14 days. Remarkably, the PMEG diphosphate levels increased for 10 days and then declined gradually. These studies suggest that infrequent topical dosing with ODE-Bn-PMEG could fill an important unmet need as a treatment for cervical and anogenital high-risk HPV infections.

153 Inhibition of herpes simplex virus type 1 (HSV1) by the CDK6 inhibitor PD-0332991 (palbociclib) through the control of SAMHD1

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¹*AIDS Research Institute-IrsiCaixa, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain,*

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Sterile a motif and HD domain-containing protein-1 (SAMHD1) has been shown to restrict retroviruses and DNA viruses by decreasing the pool of intracellular deoxynucleotides. SAMHD1 is, in turn, controlled by cyclin-dependent kinases that regulate cell cycle and cell proliferation. Mitogenic stimuli drive cells from G0 into G1 phase by inducing the expression of D-type cyclins, which activate CDK4 and CDK6. Here, we explore the effect of CDK6 inhibitors on the replication of herpes simplex virus type 1 (HSV-1) in primary monocyte derived macrophages (MDM).

Human primary monocytes were differentiated into macrophages with monocyte-colony stimulating factor. Cells were treated with palbociclib (PD0332991) and then infected with a green fluorescent protein-expressing HSV-1. Deoxynucleotide triphosphate (dNTP) content was determined using a polymerase based method.

Palbociclib, a potent and selective CDK6 inhibitor, blocked SAMHD1 phosphorylation, intracellular dNTP levels, and HSV-1 replication in primary macrophages at subtoxic concentrations. Treatment of macrophages with palbociclib led to reduced CDK2 activation, measured as the phosphorylation of the T-loop at the Thr160. The antiviral effect was lost when SAMHD1 was degraded by Vpx. Similarly, palbociclib did not block HSV-1 replication in SAMHD1 negative Vero cells at subtoxic concentrations, providing further evidence for a role of SAMHD1 in mediating the antiviral effect.

Our results indicate that SAMHD1-mediated virus restriction is controlled by CDK and point to a preferential role for CDK6 and CDK2 as mediators of SAMHD1 activation. Similarly, the restricting activity of SAMHD1 on DNA viruses suggests that control of dNTP availability is the major determinant of its antiviral activity.

154 Relationship between mutations in E2 proteins and their response to interferon alpha 2b plus ribavirin therapy in HCV genotype 3a Pakistani patients

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¹Quaid-i-Azam University, Islamabad, Pakistan, ²Nuclear Medicine, Oncology and Radiotherapy Institute, Islamabad, Pakistan

Hepatitis C Virus (HCV) is rapidly emerging as a serious health threat to humanity around the globe. E2 is a structural protein present on the viral membrane. It functions as a host receptor binding protein. We explored the relationship between mutations in E2 proteins with reference to their response to interferon alpha 2b plus ribavirin therapy in HCV genotype 3a patients. E2 region was amplified by region specific primers followed by sequencing. In responders, mutant category belongs to age group 37+10 while in non responders, mutant category belongs to age group in 48/+7. It was found that E2 protein in local HCV 3a variants had mutations and was not totally conserved. Phylogenetic analysis showed that newly reported sequences of the study had homology with the sequences from United Kingdom.

155 Antiviral Activity of a G-quadruplex Ligand Against Herpes Simplex Virus-1

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¹Department of Molecular Medicine, University of Padua, Padua, Italy, ²Department of Microbiology, Infectious Diseases and Immunology, University of Laval, Quebec, Canada

G-quadruplexes (G-4s), non-canonical secondary structures formed by G-rich nucleic acids, are thought to play crucial regulatory functions in the genomes of prokaryotes, humans and viruses.

Given the high amount of GC bases within the genome of the herpes simplex virus-1 (HSV-1), we investigated both the presence of G-4 forming sequences at the viral genome level and the possibility to target them with G-4 ligands to obtain anti-HSV-1 effects with novel mechanisms of action. Six clusters of repeated sequences, mainly located in the inverted repeats of the HSV-1 genome, were identified and proved to form very stable G-4 structures.

A G-4 ligand displayed a remarkable inhibitory effect against HSV-1. In contrast to a different anti-herpetic G-4 ligand previously tested by our group, the compound did not affect intracellular viral DNA levels, while it markedly impaired release of mature infectious viral particles from the cytoplasm of the host cell. Viral particles were shown to be entrapped within autophagosome-like vesicles in the cytoplasm of infected and treated cells. Therefore, we hypothesized that the G-4 ligand targeted HSV-1 maturation/ egress and/or cell autophagy, through an unusual G4-mediated mechanism of action. Indeed, one G-4-forming cluster was found within the promoter region of the multifunctional protein γ 134.5, which has been reported to be involved in both mechanisms. We reasoned that stabilization of the identified G-4 by the ligand could affect expression of the γ 134.5 protein, therefore stimulating cell autophagy and inhibiting viral maturation. Indeed, we found that co-treatment with an autophagy inhibitor was able to reverse the antiviral effect of the G-4 ligand.

This work provides evidence of the activity of G-4 ligands against HSV-1 and highlights the possibility to target different viral pathways through the use of singular G-4 ligands. Identification of small molecules able to selectively recognize viral G-4s versus the eukaryotic structures will provide new important tools for the treatment of HSV-1.

156 Chemokine (CCL2, CXCL10) and Interleukin (IL28B, IL10) Gene Variability and Human Predisposition to Tick-Borne Encephalitis

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Tick-borne encephalitis (TBE) is caused by single-stranded RNA virus from the *Flavivirus* genus (Flaviviridae family). Genetic predisposition to TBE is rather poorly studied in human population. Human genes encoding crucial components of antiviral immune response are most likely involved in protective mechanisms against TBE virus. Previously, mRNA levels of chemokine ligand 2 (CCL2) and interferon- γ -inducible protein 10 (CXCL10) were shown to significantly differ between mouse lines with different sensitivity to TBE virus. Several single nucleotide polymorphisms (SNPs) located in interleukin 28B (*IL28B*) and interleukin 10 (*IL10*) genes were previously associated with predisposition to chronic hepatitis C (caused by structurally similar virus from the same family) in a number of populations. In our study, genotype and allele frequencies for several SNPs in the *CCL2*, *CXCL10*, *IL28B*, and *IL10* genes were compared in 132 non-immunized TBE patients (34 with fever, 60 with meningitis, and 38 with severe forms) and in the control Russian population (221 Novosibirsk citizens). No association between the *CCL2* and *CXCL10* gene SNPs and predisposition to TBE was detected. For the *IL28B* gene rs12980275 (A/G) SNP located in 3'-flanking region, a significant increase in A/A homozygote frequency for TBE patients (60.6%) (especially those with severe disease (71.4%)) as compared with the control group (47.5%) ($P = 0.018$ and 0.009) was detected. An increase in A allele frequency for the same SNP was also found in TBE patients (77.2%) (and in patients with severe disease (84.3%)) as compared with controls (65.8%) ($P = 0.002$ and 0.002). For the *IL10* gene rs1800872 (C/A) SNP located in promoter region, no significant differences were detected between TBE patients and controls; however, an increase in A/A homozygote frequency for patients with severe disease (10.5%) as compared with the control group (2.0%) was found ($P = 0.007$). Our data suggest that the *IL28B* gene rs12980275 and *IL10* gene rs1800872 SNPs are associated with predisposition to TBE in Russian population. This work was supported by the Russian Foundation for Basic Research (grant 14-04-00641a).

157 Biochemical characterization of the PA, PA-X and the natural PA-X deletion mutant of human Influenza A virus

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The endonuclease activity of the PA subunit of the RNA dependent RNA polymerase of influenza virus is required for the cap snatching mechanism essential for the synthesis of viral mRNA. Recently, a novel protein, named PAX, has been discovered to be translated from an alternative open reading frame within the PA gene coding sequence. The PAX protein has the N-terminal endonucleolytic domain identical to PA, but has a different and shorter C-terminal domain. It has been proposed that PAX modulates viral pathogenesis in the host. In human isolates from the 2009 pandemic H1N1 virus, a naturally truncated form of PAX is present. Here we show for the first time that PAX has a robust endonucleolytic activity which has a distinct substrate specificity with respect to PA. In addition, we show that the deleted form of PAX has lost most of its enzymatic activity, due to a substrate binding defect. Our results will be discussed in light of the distinct roles of PA and PAX in the viral life cycle and their suitability as antiviral targets.

158 Non-Invasive Topical Immunization Using Cholera Toxin As Adjuvant for the treatment of Hepatitis B

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The search for innovative ways of vaccination has intensified recently with declining vaccine coverage and growing public concern about new virulent disease outbreaks. Immunization is a prophylactic approach through which the body is shielded from any incoming pathogenic invasion. Skin is potentially rich site for immunization. Immune response elicited by Topical Immunization depends upon structure and composition of the skin of target species. It provides access to local skin immune system which is dominated by langerhans cells that can be manipulated by adjuvants to orchestrate specific, robust immune response. Topical vaccination induces systemic and mucosal antibodies to the co-administered antigen and moreover it avoids the first pass phenomenon and also protects the antigen from enzymes that are present in gut wall.

Niosomes are nonionic surfactant based vesicles that can be utilized as a topical carrier for immunogens for dermal or transdermal delivery. The goal of the present study was to investigate the potentials of niosomes as carrier for Hepatitis-B antigen (HbsAg) with cholera toxin (CTB) as adjuvant.

Niosomes containing HBsAg & CTB were prepared by Sonication. Antigen loaded Niosomes were characterized *in-vitro* for their shape, size, percent antigen entrapment and stability in various body fluids. *Confocal laser scanning microscopy (CLSM)* was carried out to confirm the uptake of Niosomes. The *in-vivo* part of the study comprised of immunization of female Balb/c mice and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Niosomes formed were multilamellar and were found to be stable. Presence of fluorescence at different skin depths reflected accumulation of these niosomes in the region of epidemis, suggesting better uptake of antigen by langerhans cells. Based on the results obtained, niosomes presented its potential for antigen delivery through transcutaneous route.

159 COMBINING HEPATITIS B SURFACE ANTIGEN WITH TETANUS FOR A SINGLE ORAL VACCINE

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Infections are still a leading cause of morbidity and mortality and most of which can be prevented by vaccination. However, there are too many vaccines to be administered, increasing the cost of immunization. Combination vaccines can be an answer to these problems till the development of a single vaccine containing all the possible antigens.

The goal of the present study was to see the effect of 2 antigens when given in combination. Oral immunization induces both mucosal and systemic immune responses, whereas mucosal responses are not generally observed following systemic immunization. Bilosomes can provide needle free, painless approach for immunization, thereby increasing patient compliance and consequently increasing vaccination coverage. Recombinant hepatitis B surface antigen (HBsAg) and recombinant protective antigen (rPA) were chosen as the candidate antigens.

Bilosomes containing rPA and HBsAg were prepared by lipid cast film method. Antigen loaded bilosomes were characterized *in-vitro* for their shape, size, percent antigen entrapment and stability in various body fluids. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The *in-vivo* part of the study comprised of immunization of female Balb/c mice and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Bilosomes formed were multilamellar and were found to be stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by the gut associated lymphoid tissues. *In-vivo* data demonstrates that combination produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect.

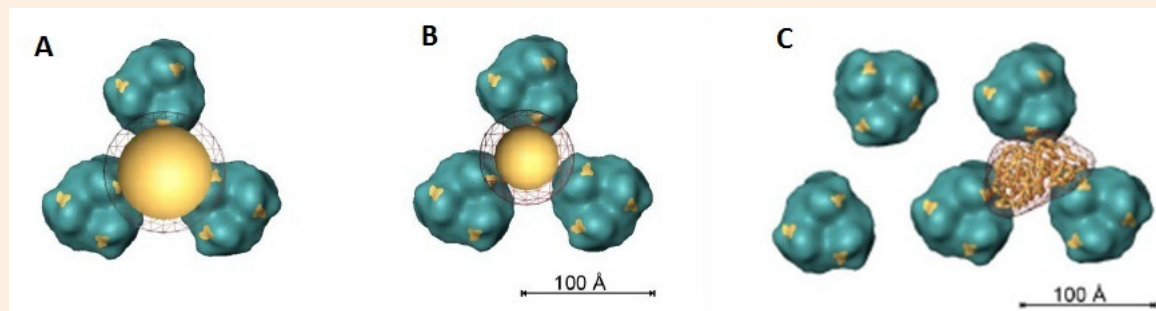
When rPA(AB) and HBsAg(AV) given in combination, HBsAg (high dose) potentiates the production anti-rPA antibody. This can be due to antigen competition or other unknown mechanisms for which exhaustive studies may be performed. Also they elicited measurable sIgA in mucosal secretions, while alum adsorbed antigens failed to elicit such responses. The combination of antiviral and antibacterial loaded bilosomes produced both systemic as well as mucosal antibody responses upon oral administration.

160 Linear and hyperbranched polyglycerol based multivalent glycoarchitectures as influenza virus inhibitors

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The topology of the carrier polymeric backbone has a great influence on the activity of multivalent competitive virus binding inhibitors. This influence can be analyzed by using different polymeric carrier backbones having the same chemical construct but different topology. A detailed analysis of three different series of sialic acid (SA) conjugated linear and hyperbranched polyglycerols as competitive influenza virus-cell binding inhibitors lead to some interesting observations which can also be further used for designing multivalent competitive inhibitors against different pathogens. An optimum degree of SA conjugation was observed for the most potent nanomolar inhibitors in the three different series as screened by cell binding inhibition and infection inhibition assays using X-31/H3N2 influenza A virus strain. The theoretical evaluation of experimental results also leads to the existence of an optimum degree of SA conjugation for the maximum inhibitory activity. The lower molecular weight IPGSA were found to be more potent than the higher molecular weight hPGSAs in terms of the weight of the inhibitor required for the significant inhibitory effect. To further understand the effect of rigidity and flexibility, glycol-inhibitors with different flexibility were designed and synthesized in the size range matching with the virus.



161 TAOK3 Phosphorylates the Methylene-cyclopropane Nucleoside MBX 2168 to its Monophosphate

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A number of methylenecyclopropane nucleosides (MCPNs) with ether or thioether substituents at the 6-position show promise as broad spectrum anti-herpes agents, as they inhibit herpes simplex virus (HSV)-1 and 2, varicella-zoster virus (VZV), human cytomegalovirus (HCMV), human herpes virus type 6 (HHV-6), human herpes virus type 8 (HHV-8), and Epstein Barr virus (EBV). These compounds are analogs of the dihydroxymethyl MCPN, cyclopropavir, which is currently being tested in a Phase I clinical trial for the treatment of HCMV infections. The proposed mechanism of action of these compounds, inhibition of the viral DNA polymerase, requires sequential phosphorylation to a triphosphate. The inhibition of HSV by these compounds is not dependent on the viral thymidine kinase (TK), which is known to phosphorylate acyclovir, a standard treatment for HSV infections. Previous studies on the mechanism of action of these compounds against HCMV implicated a host kinase in addition to HCMV UL97 kinase in performing the initial phosphorylation. After first eliminating other candidate HSV-1 encoded kinases (UL13 and US3), as well as potential host nucleoside kinases, we have now identified, through activity based fractionation, the host serine-threonine protein kinase TAOK3 as the kinase responsible for transforming the representative monohydroxymethyl MCPN analog MBX 2168 to its monophosphate.

162 Antiviral activity of AGMA1 polymer against Human Papillomaviruses and preclinical study as a topical microbicide

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Human papillomavirus (HPV) are widespread human pathogens and many types are sexually transmitted causing anogenital lesions including cancers. Indeed some viral types are highly oncogenic and therefore are named “high-risk HPV” (e.g. types 16, 18, 31, 45). HPV is the only etiological agent of cervix carcinoma which caused 266 000 deaths in 2012. Currently two vaccines are available: Gardasil and Cervarix, but even in the era of HPV vaccination, effective inhibitors of HPV infection are required particularly in low resource settings where there is the highest burden of HPV infection.

The aim of this work was to assess the antiviral potency and the spectrum of activity of an amphoteric polyamidoamine, AGMA1 against a panel low-risk and high-risk HPV and to elucidate its mechanism of action. AGMA1 was found to be a potent inhibitor of mucosal HPV types (i.e., types 16, 31, 45, 6) in pseudovirus-based neutralization assays. The 50% inhibitory concentration was between 0.34 µg/ml and 0.73 µg/ml and no evidence of cytotoxicity was observed. AGMA1 interacts with immobilized heparin and with cellular heparan sulfates exerting its antiviral action by preventing virus attachment to the cell surface. Furthermore AGMA1 shows a good biocompatibility profile on vaginal lactobacilli and on human cervicovaginal histo-cultures along with a good stability in vaginal fluid. The same compound has proved to be effective against HSV-2 in vitro, on human cervicovaginal histo-cultures and in vaginally infected mice. The findings from this study indicate AGMA1 to be a leading candidate compound for further development as an active ingredient of a topical microbicide against HPV and other sexually transmitted viral infections.

163 Efficacy of replication-defective lymphocytic choriomeningitis virus vectors (rLCMV) expressing guinea pig cytomegalovirus antigens against congenital cytomegalovirus infection in guinea pigs

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BACKGROUND: Congenital cytomegalovirus (CMV) infection can be life-threatening and often results in significant developmental deficits and/or hearing loss. Thus, there is a critical need for an effective anti-CMV vaccine.

OBJECTIVE: To determine the efficacy of replication-defective LCMV vectors expressing the GPCMV antigens, gB and pp65, in the guinea pig model of congenital CMV infection.

METHODS: Female Hartley strain guinea pigs were divided into three groups: Buffer control group (n=9), rLCMV-gB group (n=11), and rLCMV-pp65 (n=11). The vaccines were administered three times IM at 1.54×10^6 ffu per dose at 21-day intervals. At two weeks after vaccination, the female guinea pigs underwent breeding. Once pregnant, the guinea pigs were challenged SQ at ~45-55 days of gestation with 1×10^5 pfu of GPCMV. Viremia in the dams, pup survival, weights of pups at delivery, and viral load in both dam and pup tissues were determined.

RESULTS: Pup survival was significantly increased in both vaccine groups, 23% mortality in the gB vaccine pups (p=0.025) and 26% in the pp65 pups (p=0.032) compared to control pups (51% mortality). The gB vaccine group also had higher mean time to delivery and pup weights. The gB vaccine induced high levels of gB and neutralizing antibodies, reduced dam viremia, and significantly reduced viral load in dam tissues compared to control dams (p< 0.03). Reduced viral load in pups born to gB-vaccinated dams was observed compared to pups from pp65-vaccinated (p=0.08) or control dams (p=0.60).

CONCLUSIONS: Both the rLCMV-gB and rLCMV-pp65 vaccines improved pup survival while the gB vaccine also improved gestation time and pup weights. The gB vaccine was also more effective at decreasing viral load in dams and pups. Thus, rLCMV vectors that express CMV antigens may be an effective vaccine strategy for congenital CMV infection.

164 **Assessment of lethality of 1976 Ebola-Zaire and 2014 Ebola-Liberia strain in a novel humanized mouse model**

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The ongoing EbolaVirus(EBOV) outbreak in West Africa is so far the largest and already made a devastating impact on human life and socio-economic aspects of countries like Guinea, Liberia, Sierra Leone and Nigeria with 8700 human casualties till date (as of Jan30, 2015). Robust animal models that faithfully recapitulate key human disease correlates are critical to gain insights into virus pathogenesis. EBOV isolates derived directly from human specimens are lethal to non-human primates, but unfortunately do not cause disease in immune competent adult mice without mutation of key virulence determinants. We sought here to investigate if mice engrafted with human immune system components could overcome these limitations and be infected with isolates of either Ebola virus -Zaire Mayinga 1976 and Ebola virus-Liberia 2014 derived from fatal human cases. We found that these mice developed lethal disease characterized by high virus loads, alterations in key human antiviral immune gene regulation patterns, and histopathology similar to that observed in fatal human patients following infection. Engraftment of the human cellular immune system was essential for the observed virulence as non-engrafted mice did not support productive virus replication nor develop clinical disease following infection. Humanized mice may prove useful to explore the basic molecular and immunologic mechanisms that mediate interspecies barriers to Viral Hemorrhagic Fever infection and elucidate specific human factors that underlie the high case fatality of Ebola -Zaire and Ebola-Liberia strain.

165 **+Ve approach formula with four prong attack on Ebola virus infection with Antivirals, innate immune booster, anti-cytokines and vaccines along with the Antivirals passive prophylaxis to stamp out the epidemic**

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With pessimistic and negative attitude in treating Ebola virus infection without targeting the virulent virus which begets cytokine excess or storm; precipitating multi-organ failure is regrettable. This prompted us to formulate a four pronged attack; the first approach is targeted four antiviral on to the Ebola virus. The broad spectrum antiviral agent Ribavirin 200mg QID oral will be best suited since it had been used in all cell line studies as positive control. Three more anti Ebola viral drugs studied and proved in cell line studies and in animal experiments shown to block the entry of virus after binding to cell receptors and then replication; are Chloroquine [Peter B. Madrid et al 2013] 5 to 20mg/kg oral / intramuscular; clomiphene and toremiflon [Lisa M. Joyanis et al 2013] 60mg/kg oral and were approved by FDA and repurposed and so can be straight away utilized for treating Ebola virus infection. The second approach is T.Nitazoxanide 500 b.d. oral which is a small molecule antiviral agent which modulates host antiviral pathway as interferon immune enhancer [EB Keefee & J.F. Rossignol 2009] The third prong attack is a combination of anti-cytokines; again Chloroquine [Jang et al 2006], Doxycycline [Castro J.E et al 2011] shown to have actions against IL1B and 6 and TNF- and Monteleukast blocking cysteinyl receptor. Fourth and final attack by the emerging and promising vaccines.

We are in a desperate situations and four prong attack with poly pharmacy is essential and mandatory and is in a way implementing old saying of "BENCH TO BEDSIDE". The new antivirals should be administered as prophylactic treatment for all the contacts and medical and paramedical personale. The therapy protocol to be initiate from "DAY ONE OF DIAGNOSIS" to get the full benefit of the therapy by controlling both the virus replication and cytokine formation and escalation.

166 An Ethnomedicinal Alkaloid and its Analogue, inhibit Immediate Early Transcription of Herpes Simplex Virus

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Herpes simplex virus (HSV) is one of the serious public health concerns. Following entry, HSV establishes primary infection at entry site and finally transported to the sensory ganglia for life-long latency. During immediate early (IE) transcription viral tegument protein VP16 form a complex with the host cell factors HCF1 and Oct1, which requires the recruitment of LSD1 to bind with ICPO promoter for transcriptional activation, leading to the synthesis of early and late genes. The herpesvirus diseases are successfully managed by acyclovir, but its long-term clinical use leads to the emergence of drug-resistant viruses. Till date there is no effective vaccine or drug that can eliminate the virus, thus, cost-effective new agents are necessary.

We have isolated an alkaloid harmaline (HM, CC₅₀ 30µg/ml) from the ethnomedicinal herb *Ophiorrhiza nicobarica* with potent antiviral activity against HSV-1 and HSV-2 (EC₅₀ 1.1-1.5 µg/ml) by blocking the recruitment of LSD1, leading to the inhibition of viral IE gene synthesis. To reduce the toxicity of HM we have synthesized analogues, one of which containing a fused octahydro-benzo [d] imidazole (HC-II-21) showed good anti-HSV activity (EC₅₀ 26-29.5 µg/ml) with (CC₅₀ 275 µg/ml). Further lead optimization study yielded its salt form AK-IV-14-T (EC₅₀ 30.4-35.2 µg/ml) with better solubility and reduced toxicity (CC₅₀ 390 µg/ml). This presentation will discuss how HM and its analogue interference in the binding of IE complex, an interesting target for developing non-nucleotide antiherpetic agent with different mode of action than acyclovir.

167 Identification of a broad-spectrum inhibitor of common RNA respiratory viruses by high-throughput screening

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Respiratory viral infections (RVI) pose a major burden to public health. Acute viral infections cause an estimated 3.9 million deaths annually, making it one of the top five causes of mortality worldwide. Common causes of RVIs include the respiratory syncytial virus (RSV), influenza virus A (IVA) and rhinovirus (RV). Enterovirus 71 (EV71) is a lesser common cause of RVIs, but is the causative agent of hand-foot-and-mouth-disease with risk of progression to the central nervous system. Our aim was to identify broad-spectrum inhibitors of common RVIs. Previous screening of 50,240 structurally diverse compounds led to the identification of multiple inhibitors of IVA infection. These were further screened against RSV, RV and EV71. Compound FA-613 is a non-toxic compound with an EC₅₀ of 2.4 µM and 3.9 µM against IVA and EV71 respectively determined by plaque reduction assays and viral cytopathic effect observation indicates complete inhibition of RV and RSV at 10 µM. Results of time-of-addition assays with IVA showed strongest inhibition of infection when the compound was added within 4 hours after infection, suggesting that replication and transcription might be targeted. Inhibition of vRNP function was further confirmed by minigenome assays. A similarity search revealed that FA-613 is structurally related to brequinar, a known inhibitor of the pyrimidine synthesis pathway, which targets the human dihydroorotate dehydrogenase (DHODH). Broad-spectrum antiviral activity of DHODH inhibitors has been mainly attributed to the depletion of nucleosides necessary for replication of the viral genome. Excess uracil, which promotes pyrimidine salvage, indeed restores viral infection in presence of FA-613. To our knowledge, inhibition of IVA, RSV, RV and EV71 by a single compound has not been described before. Since FA-613 is targeting a host pathway, the emergence of resistant viruses is not expected. Therefore, in acute RVIs, when rapid diagnostics of the causative agent are not readily available, an antiviral drug with properties like FA-613 could be very valuable.

168 Protective efficacy of VP1-specific neutralizing antibody associated with a reduction of viral load and pro-inflammatory cytokines in human SCARB2-transgenic mice

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Hand-foot-mouth diseases (HFMD) caused by enterovirus 71 (EV71) and coxsackievirus 16 (CVA16) in children has now become a severe public health issue in the Asian-Pacific region. Recently we have successfully developed transgenic mice expressing human scavenger receptor class B member 2 (hSCARB2, a receptor of EV71 and CVA16) as an animal model for evaluating the pathogenesis of enterovirus infections. In this study, hSCARB2-transgenic mice were used to investigate the efficacy conferred by a previously described EV71 neutralizing antibody, N3. A single injection of N3 effectively inhibited the HFMD-like skin scurfs in mice pre-infected with clinical isolate of EV71 E59 (B4 genotype) or prevented severe limb paralysis and death in mice pre-inoculated with 5746 (C2 genotype). This protection was correlated with remarkable reduction of viral loads in the brain, spinal cord and limb muscles. Accumulated viral loads and the associated pro-inflammatory cytokines were all reduced. The protective efficacy of N3 was not observed in animals challenged with CVA16. This could be due to dissimilarity sequences of the neutralizing epitope found in CVA16. These results indicate N3 could be useful in treating severe EV71 infections and the hSCARB2-transgenic mouse could be used to evaluate the protective efficacy of potential anti-enterovirus agent candidates.

169 TELAPREVIR-INDUCED DRUG RASH WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS ASSOCIATED WITH HHV-6 ACTIVE INFECTION.

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A 51-year-old woman with chronic hepatitis C received interferon and ribavirin for 4 weeks. Then Telaprevir was added in absence of skin lesions and after three weeks HCV plasma viremia was undetectable. Two weeks later, physical examination revealed diffuse skin eruption, oro-pharyngeal hyperemia and diffuse lymphadenopathies with pruritus, fever and arthralgias. Laboratory findings showed eosinophilia and lymphocytopenia. Antibodies against Cytomegalovirus, Epstein Barr Virus, Adenovirus indicated past infections. Plasma HCV-RNA remained negative. Anti-HHV-6 IgG antibodies were positive, IgM negative. HHV-6 systemic reactivation was demonstrated in serum by HHV-6 viremia and in skin tissue by quantitative Real-time Polymerase chain reaction assay and immunohistochemistry. A skin histopathological examination confirmed a drug reaction and diagnosis of DRESS was made for the presence of the 7 diagnostic criteria established by the Japanese Reasearch Committee on Severe Cutaneous Adverse Drug Reaction: skin rash developing at least 3 weeks after starting therapy, prolonged clinical symptoms after drug discontinuation, lymphadenopathy, fever, leukocyte and liver abnormalities, HHV-6 reactivation. Telaprevir was discontinued and oral prednisone started. Cutaneous and systemic symptoms rapidly improved and the blood cells count returned within normal value in two weeks. Prednisone was gradually decreased and stopped in one month. Sixteen other cases of Telaprevir-induced DRESS have been described from 2010 to date but without mention on HHV-6 reactivation. We emphasize the increasing risk for DRESS in Telaprevir-treated patients requiring attention for the possible life-threatening evolution.

170 Ribonuclease H/DNA polymerase HIV-1 RT dual inhibitor: mechanistic studies on the allosteric mode of action of isatine-based compound RMNC6

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The human Immunodeficiency virus (HIV) reverse transcriptase (RT) has been the most pursued target for antiretroviral therapy. RT performs viral genome retrotranscription by two associated functions: DNA polymerase and ribonuclease H (RNase H), both of them validated targets for drug discovery. Different classes of compounds have been reported to inhibit both RT functions, supporting the possibility to inhibit both functions with one molecule possibly: i) targeting new allosteric RT sites, ii) being effective on known drug resistant variants and iii) minimizing the selection of new drug resistant variants. We recently identified a new isatine-based scaffold for dual RT inhibitors. The most active compound, RMNC6 (IC₅₀ values of 1.4 and 9.8 μM on RNase H and DNA polymerase activities, respectively), was chosen to investigate its mode of action. A first blind docking study on wt RT suggested that RMNC6 could bind to two different RT pockets: one on the polymerase domain, partially overlapping the non-nucleoside RT inhibitors

(NNRTI) binding pocket and another close to the RNase H active site. This hypothesis has been investigated by a combination of kinetic and site directed mutagenesis studies. On the one hand, RMNC6 showed to be not kinetically mutually exclusive with Efavirenz, even though it negatively interfered with its binding to RT, suggesting that RMNC6 could bind to a site different from the NNRTI pocket. Consistently, RMNC6 retained full potency on both RT functions when tested on a naturally NNRTI resistant RT (HIV-1 O subtype). On the other hand, site-directed mutagenesis showed that amino acid residues N474, Y501, A502 and A508 in the RNase H domain are important for RMNC6 inhibition of the RNase H function but not for its DNA polymerase inhibition. Overall, these results support the hypothesis that RMNC6 could bind to two different RT sites: one in the RNase H domain, responsible for RNase H inhibition, one in the DNA polymerase domain, affecting the RT-associated DNA polymerase function.

171 Optimization of Simeprevir Triple Therapy: A Multivariate Logistic Regression Model Using Baseline Predictors

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AIM: To optimize treatment with simeprevir (SMV) + pegylated-interferon/ribavirin (PR) we examined predictors of response using a multivariate logistic regression model. The effect of including RVR and Week 4 response in the model was also examined.

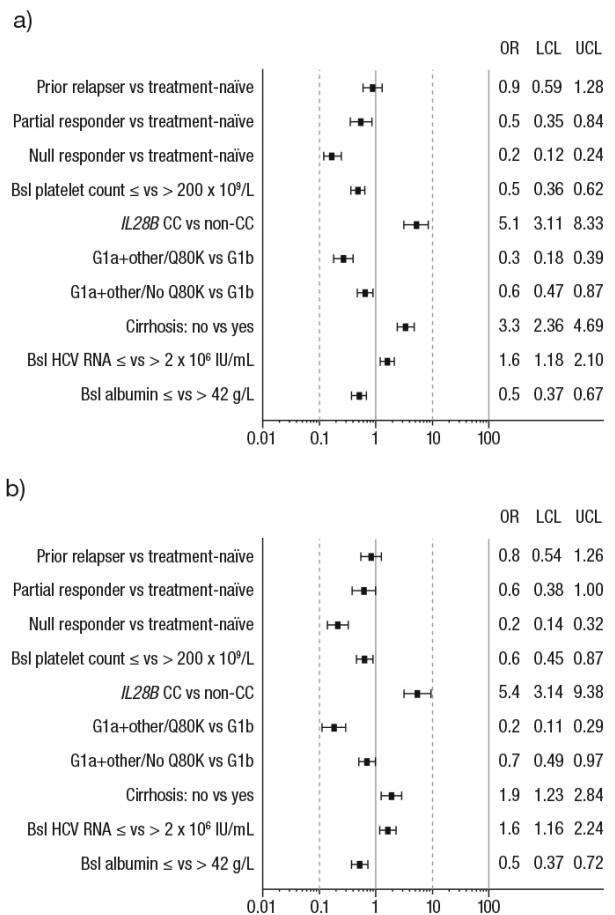
DESIGN: Data from patients treated with SMV+PR in four Phase III studies were included: QUEST-1, QUEST-2, ATTAIN and PROMISE. Univariate/multivariate selection procedures identified prognostic factors of SVR. These were used to produce a multivariate logistic regression model to predict response for individual patients. Goodness-of-fit tests assessed the robustness of the model.

RESULTS: Data comprised 1062 patients (45% G1a+other / 55% G1b; 44% PR-naïve / 23% PR-relapsers / 33% PR-non-responders; 20% *IL28B* CC; 15% cirrhotic). A higher likelihood of achieving SVR was associated with several baseline factors: absence of cirrhosis, *IL28B* CC, absence of Q80K in G1a, treatment-naïve or prior relapser patients, lower HCV-RNA, higher albumin or platelets (Figure). RVR was not included as a factor in this analysis.

The multivariate model comprising these factors was confirmed a good fit (AUC: 0.79). The fit improved when RVR was added as a covariate to the model (AUC: 0.84).

CONCLUSIONS: The model allowed prediction of SVR for patients being considered for SMV+PR therapy; this may prove useful in settings where access to IFN-free therapy is limited or unavailable. The model also confirms the importance of RVR in predicting SVR. SMV+PR was well tolerated with a similar adverse event profile to that of PR alone.

Figure: Baseline (Bsl) predictors of SVR identified by a) univariate and b) multivariate analyses with RVR excluded



172 Identification of FDA-approved drugs that inhibit Middle East Respiratory Syndrome Coronavirus replication in cell culture

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In addition to the 2003 epidemics of severe acute respiratory syndrome (SARS), also the recent outbreak of the Middle East respiratory syndrome coronavirus (MERS-CoV) demonstrates the potentially lethal consequences of zoonotic coronavirus infection in humans. Thus far, its fatality rate (~35%) among >900 laboratory-confirmed human cases is alarmingly high, even though many deaths were associated with underlying medical conditions. Ten years after the 2003 SARS outbreak, registered drugs to treat coronavirus infections are still not available. Therefore, we set out to develop a cell culture-based assay to screen for antivirals against MERS-CoV, based on the pronounced cytopathology the virus causes in Huh7 and Vero cells. This assay in a 96-well format was initially used to confirm cyclosporin A and interferon- α as inhibitors of MERS-CoV replication in cell culture (De Wilde et al., J. Gen. Virol., 2013). We next screened a library of ~700 FDA-approved compounds for their antiviral activity against MERS-CoV. Six compounds, including Chlorpromazine HCl, Chloroquine, Loperamide HCl, and Lopinavir, were found to significantly inhibit MERS-CoV replication with EC₅₀ values in the low-micromolar range. These compounds were also found to inhibit the replication of two other human coronaviruses, *i.e.* SARS-CoV and human CoV-229E, with comparable efficacy (De Wilde et al., AAC, 2014). Their mode of action is currently being investigated, after which the most potent compounds will be subjected to further testing. This will include analysing the antiviral effect of combinations of FDA-approved compounds with e.g. previously reported MERS-CoV inhibitors like interferon alpha to lower the effective dose. Evaluation in a MERS and/or SARS-CoV small-animal model will be included to assess whether (combinations of) these compounds indeed broaden the therapeutic options to combat MERS or human coronavirus infections in general.

173 Identification of highly conserved residues involved in the inhibition of the HIV-1 integrase by diketoacid derivatives

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HIV-1 Integrase (IN) active site inhibitors are the latest class of drugs approved for anti HIV-1 treatment and are currently investigated to find novel and more potent agents against multi-drug resistant HIV-1 strains. Diketo acid (DKA) derivatives are among the first compounds reported to interact with the Mg²⁺ cofactors within IN active site. Interestingly, some DKAs were also reported to inhibit the HIV-1 reverse transcriptase-associated Ribonuclease H (RNase H) another emerging viral target. Recently, a series of pyrrolyl DKA derivatives of the 6-[1-(4-fluorophenyl)methyl-1H-pyrrol-2-yl]-2,4-dioxo-5-hexenoic acid ethyl ester (RDS1643) was reported to inhibit the HIV-1 IN within the nanomolar range and the HIV-1 replication in cell culture (1, 2). In the present study, four promising DKA compounds were chosen as chemical tools to investigate their interaction within the IN active site and mode of action. Determination of viral DNA accumulation HIV-1 replication in early phases confirmed IN as drug target while site-directed mutagenesis coupled with molecular modeling studies confirmed the interaction of the pyrrolyl scaffold with IN amino acid residues 145, 146 and 148. Interestingly, selected derivatives were effective on HIV-1 Raltegravir resistant IN strains Y143A and N155H, indicating differences in their IN interaction pattern with respect to Raltegravir. Hence, these data provide important insights for the rational optimization of new IN inhibitors active on Raltegravir resistant variants. Furthermore, comparative modeling studies on IN and RNase H active sites allowed to better delineate the DKA structural features required to modulate the potency towards either IN or RNase H. This information might also be exploited to develop effective dual IN/RNase H inhibitors as new chemical agents against HIV-infection.

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175 Cellular promyelocytic leukemia protein is an important dengue virus restriction factor

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Promyelocytic leukemia (PML) nuclear bodies (NBs) are discrete nuclear structures that recruit many proteins involved in the regulation of several cellular functions, including antiviral response. The importance of PML as a DNA virus restriction factor has been shown previously, but little information is available regarding the antiviral role of PML against RNA viruses. Dengue virus (DENV) is an emerging mosquito-borne human pathogen included in the family *Flaviviridae* that affects millions of individuals each year by causing severe and potentially fatal syndromes. Since no licensed antiviral drug against DENV infection is currently available and the most advanced DENV vaccine candidate did not meet expectations in a recent large clinical trial, it is of great importance to understand the factors mediating intrinsic immunity which may lead to the development of new pharmacological agents that can boost their potency and thereby lead to treatments for this viral disease.

In the present study we have explored the antiviral role of PML in the *in vitro* replication of DENV-2 in human A549 cells. PML silencing and overexpression resulted in a 0.76 log increase or 1.6 log decrease in virus yield, respectively. Moreover, intracellular localization of PML-NBs during DENV-2 infection was analyzed by immunofluorescence. These studies showed that PML-NBs underwent a rearrangement from nucleus to cytoplasm during infection. The number of PML-NBs per nucleus was significantly lower in DENV-2 infected cells and, interestingly, non-infected neighboring cells showed a marked increase in PML-NBs number and size.

To gain more insight about the PML-mediated antiviral response, the level of mRNAs of IFN pathway related genes was quantified in DENV-2 infected A549 cell cultures. PML-mRNA expression level increased by 5000-fold relative to non-infected cells. Kinetics of PML-mRNA expression along DENV-2 infection were also studied, revealing that even at 2 hours post infection PML-mRNA level was upregulated by 1000-fold.

Overall, we show for the first time the contribution of PML to the cellular antiviral response against DENV-2. In order to elucidate the mechanism behind it, further studies are currently being performed.

176 Small molecule inhibitors of the HIV-1 protein Rev

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Rev is an essential regulatory protein encoded by human immunodeficiency virus type-1 (HIV-1) that allows transport of unspliced or incompletely spliced viral RNA molecules to the cytoplasm of the infected cell in the late phase of the virus cycle. The chain of events leading to viral RNA transport is triggered by a high-affinity interaction between an arginine-rich alpha-helix of Rev and an internal loop formed by the Rev Response Element (RRE), a strongly conserved nucleotide structure formed by the RNA of the virus. Although Rev represents an alternative target for HIV-1 therapy, up to now there are no Rev-based inhibitors in clinical use. We will describe the structure-based design and development of small molecule mimics of the RNA-binding alpha-helix of Rev. These compounds contain a novel bilaterally-substituted p-terphenylene scaffold that reproduces the interactions of the protein when wrapped by RNA. Cellular assays indicated that the terphenyl compounds blocked Rev function and inhibited HIV-1 replication at post-transcriptional steps of the virus infectious cycle without inducing toxicity. New data will likewise be presented regarding the transcriptional inhibition properties of these molecules, as well the identification of alternative HIV-1 inhibitors based on RRE-Rev screening. References: González-Bulnes et al. Structure-based design of an RNA-binding p-terphenylene scaffold that inhibits HIV-1 Rev protein function. *Angew. Chem. Int. Ed.* 52, 13405 (2013). Gallego et al. Bilaterally-substituted tricyclic compounds for the treatment of human immunodeficiency virus type-1 (HIV-1) and other diseases. PCT Int. application WO2014128198.

177 MP-12 lacking a functional NSs gene confers complete protection against lethal Rift Valley fever virus disease in hamster models of vaccine and post-exposure intervention**Brian B. Gowen¹**, Jonna B. Westover¹, Eric J. Sefing¹, Kevin W. Bailey¹, Luci Wandersee¹, Dionna Scharton¹, Tetsuro Ikegami²¹*Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, USA*, ²*Department of Pathology, Sealy Center for Vaccine Development and Center for Biodefense and Emerging Infectious Diseases, The University of Texas Medical Branch, Galveston, USA*

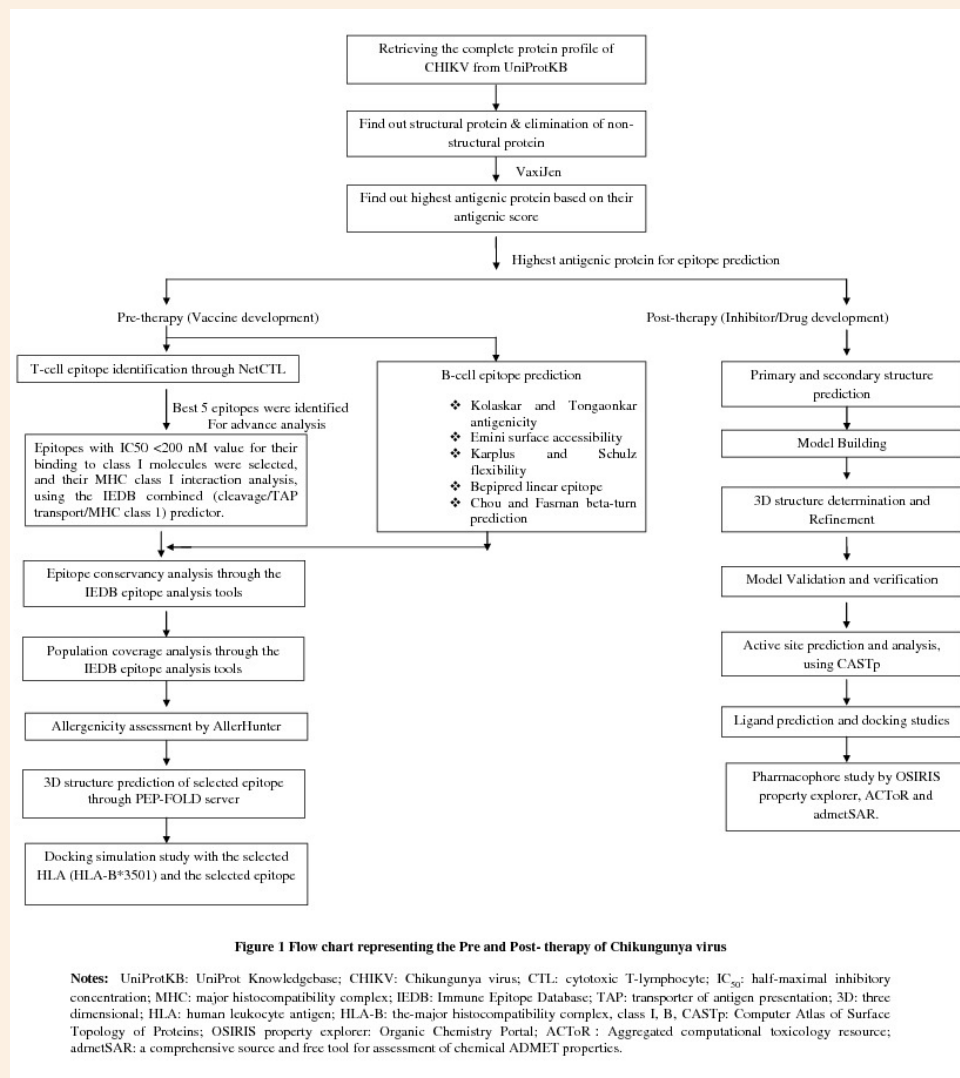
Rift Valley fever virus (RVFV; *Bunyaviridae*, *Phlebovirus*) causes a range of illnesses that include retinitis, fulminant hepatitis, neurologic disease, and hemorrhagic fever. In hospitalized individuals, case fatality rates can be as high as 10-20%. There are no vaccines or antivirals approved for human use to prevent or treat severe RVFV infections. Previously we showed that post-exposure vaccination with rMP12-C13type, a recombinant MP-12 vaccine virus which encodes an in-frame truncation removing 69% of the NSs protein, resulted in limited to moderate efficacy in mice challenged with wild-type RVFV strain ZH501, if administered within 30 minutes post-infection. Here, we demonstrate uniform protection of hamsters by post-exposure vaccination with rMP12-C13type administered 6 h post-ZH501 infection, while no efficacy was observed with the parental MP-12 virus. Notably, both the MP-12 and rMP12-C13type viruses were highly effective (100% protection) when administered 21 days prior to challenge. In a subsequent study delaying vaccination until 8, 12 and 24 h post-RVFV exposure, we observed 80, 70 and 30% survival, respectively. Our findings indicate that the rapid protective innate immune response elicited by rMP12-C13type may be due to the truncated NSs protein, suggesting that the resulting functional inactivation of NSs plays an important role in the observed post-exposure efficacy. Taken together, the data demonstrate that post-exposure vaccination with rMP12-C13type is effective in limiting ZH501 replication and associated disease in standard pre-exposure vaccination and post-challenge treatment models of RVFV infection, and suggest an extended post-exposure prophylaxis window beyond that initially observed in mice. *This work was supported by the National Institutes of Health (HHSN272201000039I).*

178 **A comprehensive immunoinformatics and target site study revealed the corner-stone towards Chikungunya virus treatment**

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Chikungunya virus (CHIKV) is a worldwide emerging threat which causes Chikungunya fever and devitalizing arthritis. Despite severe outbreaks and lack of antiviral drug, a mere progress has been made regarding to an epitope-based vaccine designed for CHIKV. In this study, whole proteome of CHIKV were retrieved from database and identify the most immunogenic protein. Structural properties of the selected protein were analyzed. The capacity to induce both humoral and cell-mediated immunity by T cell and B cell were checked for the selected protein. The peptide region spanning 9 amino acids from 397-405 and the sequence YYELYPTM were found as the most potential B cell and T cell epitopes respectively. This peptide could interact with as many as 19HLAs and showed high population coverage ranging from 69.50% to 84.94%. By using *in silico* docking techniques the epitope was further assessed for binding against HLA molecules. In addition with this, the allergenicity of the epitopes was also evaluated. In the post therapeutic strategy, 3-D structure was predicted along with validation that resulted in molecular docking study to identify the suitable therapeutic drug against targeted protein. Finally, pharmacophore study was also performed in quest of seeing potent drug activity. However, this computational epitope-based peptide vaccine designing and target site prediction against CHIKV opens up a new horizon in Chikungunya virus research.



179 **Design of potential RNAi (miRNA and siRNA) molecules for Middle East respiratory syndrome coronavirus (MERS-CoV) gene silencing by computational method**

Md.

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The Middle East respiratory syndrome coronavirus (MERS-CoV) is a virus that manifests itself in viral infection with fever, cough, renal failure and severe acute pneumonia, which often result in a fatal outcome. MERS-CoV has been shown to spread between people who are in close contact. Transmission from infected patients to healthcare personnel has also been observed and is irredeemable with present technology. Genetic studies on MERS-CoV have shown that ORF 1ab encodes replicase polyproteins and play a foremost role in viral infection. Therefore, ORF 1ab replicase polyprotein may be used as suitable target for disease diagnosis. Viral activity can be restrained through RNA interference (RNAi) technology, an influential method for post transcriptional gene silencing in a sequence specific manner. However, there is a genetic variability in different viral isolates; it is a great challenge to design potential RNAi (miRNA and siRNA) molecules which can silence the respective target genes rather than any other viral gene simultaneously. In current study four effective miRNA and five siRNA molecules for silencing of nine different strains of MERS-CoV were rationally designed and validated using computational methods, which may lead to knockdown the activity of virus. Thus, this approach may provide an insight for the chemical synthesis of antiviral RNA molecule for the treatment of MERS-CoV, at genome level.

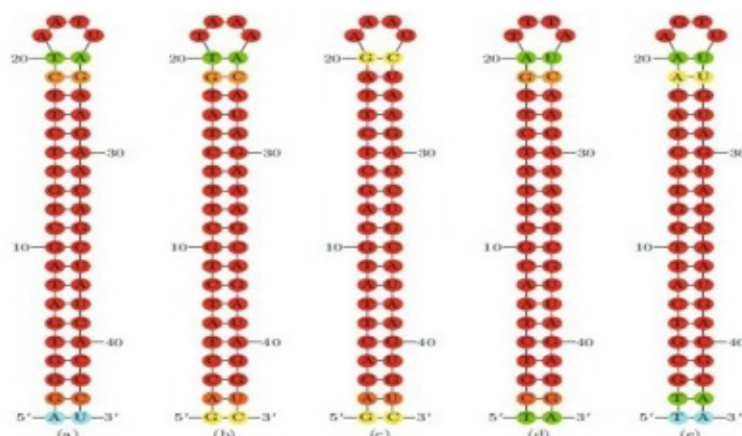


Fig. 1 (a)-(e): Secondary Structures of Target- siRNA Duplex

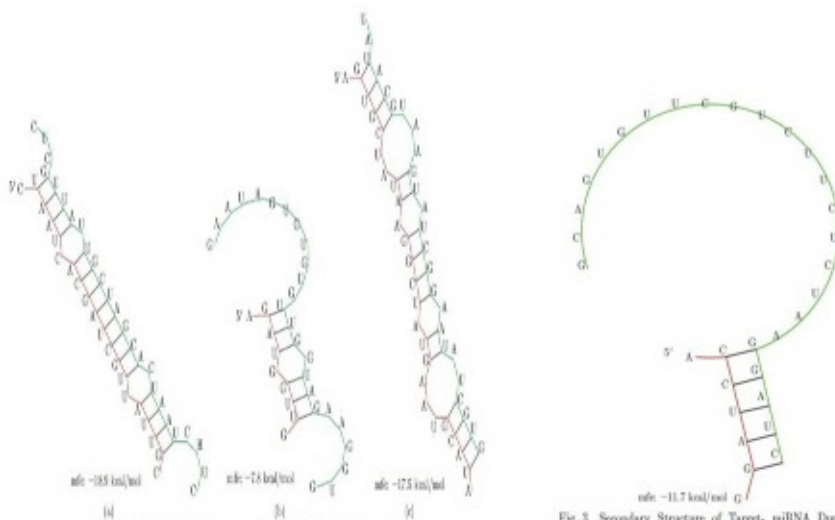


Fig. 2 (a)-(c): Secondary Structures of Target-miRNA Duplex (Group 1)

Fig. 3 Secondary Structure of Target- miRNA Duplex (Group 2)

180 Structural studies on MERS-CoV proteins: Basis for antiviral drug discovery

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The Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) is a highly pathogenic human coronavirus that was first identified in a patient from Saudi Arabia in 2012 (1,2). Since then, almost 900 cases of MERS have been recorded, most of them on the Arab peninsula; the case/fatality rate is above 30%. No vaccine or antiviral drug is available to prevent or treat infection with MERS-CoV.

Non-structural protein 3 (Nsp3) is the largest protein encoded by the MERS-CoV genome. It includes several domains, in the order Ac-X-SLD-PL^{pro}-NAB-TM-Y. The X domain of coronaviruses appears to be involved in suppressing the antiviral response of the host cell (3). The papain-like protease (PL^{pro}) is responsible for releasing Nsp1, 2, and 3 from polyproteins 1a/1ab of MERS-CoV. It also displays deubiquitinating and deISGylating activities (4-6). In order to understand functions of individual domains in Nsp3 and to target them by small-molecule compounds, it is important to elucidate the three-dimensional structures of these domains. We have determined crystal structures for the X domain and the PL^{pro}, and built a homology model for the SARS-unique domain-like domain (SLD). All three domains have been characterized functionally and the three-dimensional structures are now used to identify small-molecule inhibitors.

The most important antiviral drug target of coronaviruses is the main protease, Nsp5 (7). We have determined the crystal structure of Nsp5 of the bat-CoV HKU4, which is highly similar to MERS-CoV. Antiviral compounds designed on the basis of this structure will be presented.

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181 Human Subtilase SKI-1/S1P is a Master Regulator of the Dengue Virus Lifecycle and a Potential Target for Indirect-Acting Antiviral Agents

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BACKGROUND: Viral hijacking and manipulation of host cell biosynthetic pathways by human enveloped viruses are shared molecular events essential for the viral lifecycle. In the case of *Flaviviridae* members such as hepatitis C virus and dengue virus (DENV), one of the key host cell pathways manipulated is the lipid metabolic pathway. In this study, we hypothesize that targeting cellular enzymes acting as master regulators of lipid homeostasis could represent a powerful approach to developing a novel class of broad-spectrum antivirals against infection associated with all four DENV serotypes (1-4) found in dengue-endemic areas around the world.

METHODOLOGY/PRINCIPAL FINDINGS: Here we report that strategic manipulation of cellular SKI-1/S1P enzymatic activity by the active-site-directed aminopyrrolidineamide-based inhibitor PF-429242 provides a means of effectively inhibiting viral infection of human hepatoma (Huh-7.5.1) cells by all four DENV serotypes (1-4). SKI-1/S1P is a master regulator of the lipid homeostasis/sterol regulatory element binding protein (SREBP) pathway. We demonstrate that inhibition of SKI-1/S1P using PF-429242 results in a dose-dependent inhibition of DENV infection, pre- and post-establishment of viral infection in Huh-7.5.1 cells ($EC_{50} = 0.76$ μ M). Using plaque assays, we observed a ~3-log decrease in DENV-2 titer following pre-treatment of Huh-7.5.1 cells with 20 μ M of PF-429242 prior to infection with DENV-2. The antiviral effect of the SKI-1/S1P-directed inhibitor is associated with a robust block of SKI-1/S1P-dependent proteolytic cleavage of SREBPs in hepatoma cells.

CONCLUSIONS/SIGNIFICANCE: The results of our studies uncover the molecular function of human SKI-1/S1P in the DENV lifecycle and identify a novel host-directed and broad-spectrum antiviral target. Our studies also reveal the potential therapeutic opportunities associated with the use of lipid-modulating drugs for controlling infection of *Flaviviridae* viruses, such as DENVs, that hijack the host cell lipid metabolic pathways to support their lifecycle.

182 Effect of nucleic acid sequence on DNA polymerization and NNRTI inhibitory mechanisms of HIV-1 Reverse Transcriptase

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HIV-1 reverse transcriptase (RT) is a very well-studied enzyme, and the target of most approved antiretroviral drugs. Nonnucleoside RT inhibitors (NNRTIs) are clinically important for the treatment of HIV infection. Numerous biochemical and structural studies have provided valuable insights into the mechanisms by which NNRTIs affect the activity of HIV RT. Structural comparisons of RT in complex with various NNRTIs have revealed differences in the conformation of DNA-interacting structural regions of RT, such as the p66 thumb and the DNA primer grip. Moreover, a recent crystal structure of RT in complex with DNA and nevirapine (Das *et al.* NSMB, 19 253-9) has shown that NNRTI binding repositions the 3' end of the DNA primer and distorts the dNTP-binding site that comprises protein as well as DNA components. Hence, we hypothesized that variations in DNA sequence can modulate the efficacy of NNRTIs and their mechanism of inhibition.

To test this hypothesis, we determined the position of RT on DNA of various sequences using a site-specific hydroxyl-radical footprinting assay, in the presence and absence of different concentrations of various NNRTIs. This approach revealed surprising changes in RT-DNA binding caused by different NNRTIs, with lateral displacements of template/primers that were dependent on the nucleic acid sequences, as well as on the type of NNRTI bound. To determine whether these site-specific changes correlate with differences in the ability of RT to biochemically recognize the dNTP and DNA substrates, we performed transient-state kinetic analysis and demonstrated significant sequence-specific changes in DNA- and dNTP-binding affinities ($K_{d,DNA}$ and $K_{d,dNTP}$ respectively) and catalytic turnover (k_{pol}).

Taken together, our data suggest that (a) NNRTIs have significant effects on RT-DNA binding conformations, which are greatly influenced by the DNA sequence and the type of NNRTI, and that (b) the DNA polymerization properties of RT vary significantly with DNA sequence.

183 Development of Bipolymer based Novel Nanoparticles in Microsphere System as Vaccine Adjuvant

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Novel strategies are required for the achievement of safe and effective immunization beyond the conventional strategies. Frequent booster dosing can be avoided by the development of a mucosal/adjuvant vaccine delivery system, which can safely produce high and long lasting immune responses. Mucosal immunization is an attractive alternative to parenteral as with the appropriate delivery system it is possible to stimulate both humoral and cell-mediated responses.

The research work envisaged promotes the advantages and overcomes the disadvantages of the hydrophilic and hydrophobic polymeric systems, by a combined hydrophilic (gelatin nanoparticles, GN) with a hydrophobic polymeric system (PLGA microspheres). This combination creates a new biodegradable system for HBsAg delivery.

GN & PLGA microspheres were prepared by double emulsification method and composite system was prepared by phase separation method. Antigen loaded composites were optimized and characterized *in-vitro* for their shape, size, %antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake of composites. The *in-vivo* part of the study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. The external morphology was studied by Scanning & Transmission Electron Microscopy. The *in-vitro* studies exhibited an initial burst release from gelatin nanoparticles, degradation of antigen from PLGA microspheres & a continuous release from composite system. This supports the hypothesis to formulate single shot vaccine with such system (to mimic booster dosing). The fluorescence studies showed the selective uptake of composites by NALT.

Humoral response generated by single dose of composites was comparative to marketed formulation that received the booster dose. Further, composite system generated the effective sIgA antibody which was not elicited by the marketed formulation. Thus, it could be concluded from the present study that bipolymer based composite system are capable to provide sufficient protein stability and can be a promising candidate for development of single shot vaccine, not only against Hepatitis but against all those diseases that invade the host by the mucosal surfaces.

184 **Discovery an Antiviral Agent Targeting the Influenza Viral M2 Protein from Target-free Screening of a Chemical Library**

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A cytopathic effect-reduction assay was performed to discover anti-influenza viral agents from a chemical library composed of 2,000 existing drugs and natural products. Among the hit compounds, compound X inhibited the infection of influenza A viruses A/Puerto Rico/8/34 (PR8) (H1N1), A/Hong Kong/8/68 (H3N2) and B/Lee/40 with half maximal effective concentrations of 0.5, 0.4, and 1.6 μM , respectively. Its half maximal cytotoxicity concentration in Madin-Darby canine kidney cells was about 22.9 μM . The antiviral efficacy was further confirmed by the plaque inhibition assay and Western blot analysis, resulting in the reduction of both influenza viral plaque titers and protein expression by compound X in a dose dependent manner. Confocal microscopy showed obvious accumulation of the viral nucleoprotein (NP) in the cytoplasm of the influenza virus-infected cells at 5 h post-infection, when treated with compound X, while NP was efficiently localized into the nucleus without the compound. Measurement of proton conductance across influenza A matrix 2 protein (M2) incorporated into the lipid bilayer of virus-like particles (VLPs) suggested that compound X inhibited proton movement across the M2 channel. In conclusion, we propose that compound X potentially targets the viral entry step by blocking the proton channel function of M2.

185 **Isolation and evaluation of anti-viral activity of Trachyspermum ammi plant extract against Influenza A virus infection.**

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BACKGROUND: Influenza virus is a respiratory pathogen of global importance which causes a high degree of morbidity and mortality. Due to frequent antigenic and genetic changes, vaccines need to be formulated yearly thus requiring lot of financial investment. Naturally derived products from medicinal plants have shown great potential in preventing and/or ameliorating diseases. This study aims at evaluating the antiviral efficacy of *Trachyspermum ammi* plant extracts, having expected antiviral activity for the development of an alternative and effective therapy against Influenza A viruses. **METHODS:** Certified Plant materials were procured and extracts were prepared in ethanol. The extracts were dissolved in DMSO, aliquoted and stored in -20°C . Cytotoxicity was checked by MTT assay after 24h and 48h in A549 cells. The Anti-viral activity of the extract was assessed against Influenza A virus (A/PR/8/34-H1N1) infection by real time PCR. Plaque reduction assay was performed in MDCK cells from cell supernatant of infected A549 cells treated with plant extracts. Interferons represent a family of cytokines, which is of central importance in the innate immune response to virus infections. Modulation of Interferon stimulating genes (ISGs) were evaluated by real time PCR. **RESULTS:** A marked reduction in cytopathic effect caused by Influenza A Virus was observed post treatment *Trachyspermum ammi* in A549 cells. A similar inhibition in viral RNA (56% approx) was observed by real time PCR when treated with *Trachyspermum ammi* extracts. Relative reduction of plaques was observed in *Trachyspermum ammi* treated cell supernatant of A549 cells. **Conclusion:** Extract from *Trachyspermum ammi* showed potential inhibition of replication Influenza A virus in A549 cells.

186 **Inhibitory Effect of Peramivir against Avian Influenza A (H7N9) Virus**

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BACKGROUND: Human cases infected with avian influenza A/H7N9 virus were identified in 2013. Many laboratory-confirmed cases of human infection have been reported. However, there is few data about anti-viral treatments. Peramivir, recently approved by FDA, is an intravenous inhibitor of influenza virus neuraminidase (NA) and effective in clinically severe cases. We investigated the inhibitory effect of peramivir against A/H7N9 influenza viruses in vitro and in vivo. **Methods:** Influenza virus A/Anhui/1/2013 (H7N9) was used. To determine the sensitivity to neuraminidase inhibitors by enzyme assay using MUNANA as a substrate. In the mouse model infected with A/Anhui/1/2013, the therapeutic effect of peramivir was compared with that of oseltamivir phosphate. As a parameter for evaluation, prevention of weight loss was observed for 14 days post-virus inoculation. Virus titer and cytokine in the lungs from the mice challenged with the homologous virus were analyzed. **Results:** Peramivir showed a strong inhibitory effect on NA activity against A/H7N9 influenza virus in vitro and on weight loss of mice infected with virus in vivo. The mice showed less than 10% loss of weight with single dosing of peramivir, while mice with repetitive dosing of oseltamivir phosphate for 5 days showed around a 30% loss of weight. Five days administration of peramivir showed more effective protection. No weight change was observed by multi-dosing therapy with peramivir at 90 mg/kg. Multi-dosing therapy with peramivir significantly reduced virus titer and cytokine induction in the lungs of infected mice. **Conclusions:** These data demonstrate that peramivir has a strong inhibitory activity against A/H7N9 influenza virus.

187 Soraphen A: a Broad-spectrum Antiviral Natural Product with Potent Anti-hepatitis C Virus Activity

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BACKGROUND & AIMS: Co-infections by the hepatitis C virus (HCV) and human immunodeficiency virus (HIV) represent a significant challenge for treatment due to the necessity of combining diverse antiviral compounds that often leads to complex drug-drug interactions. Soraphen A (SorA) is a myxobacterial metabolite that inhibits the acetyl-CoA carboxylase, a key enzyme in lipid biosynthesis. We have previously identified SorA to efficiently inhibit HIV. The aim of the present study was to evaluate the capacity of SorA and analogues to inhibit HCV infection.

METHODS: SorA inhibition capacity was evaluated *in vitro* using cell-culture derived HCV, HCV pseudoparticles and subgenomic replicons. Infection studies were performed in the hepatoma cell line Huh7/Scr and in primary human hepatocytes. The effects of SorA on membranous web formation were analyzed by electron microscopy.

Results: SorA potently inhibits HCV infection at nanomolar concentrations. Obtained EC₅₀ values were 0.70 nM with a HCV reporter genome, 2.30 nM with wild-type HCV and 2.52 nM with subgenomic HCV replicons. SorA neither inhibited HCV RNA translation nor HCV entry, as demonstrated with subgenomic HCV replicons and HCV pseudoparticles, suggesting an effect on HCV replication. Consistent with this, evidence was obtained that SorA interferes with formation of the membranous web, the site of HCV replication. Finally, a series of natural and synthetic SorA analogues helped to establish a first structure-activity relationship.

CONCLUSIONS: SorA has a very potent anti-HCV activity. Since it also inhibits HIV, SorA is a promising candidate for the development of simplified treatments of HCV/HIV co-infection.

188 Host Protein Disulfide Isomerase Represents a Novel Therapeutic Target for the Treatment of Chikungunya Virus Infection

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Alphaviruses represent a broad family of pathogenic arboviruses, including New World viruses such as Venezuelan equine encephalitis virus (VEEV) and Old World viruses such as chikungunya virus (CHIKV). Alphaviruses as a genus are neglected pathogens, with an immense global disease burden but no FDA-approved vaccines or therapeutics. These viruses are composed of positive sense, single stranded RNA encased in a capsid and a host-derived envelope; embedded within this envelope are trimeric spikes of the structural E1 and E2 protein heterodimers, which are vital to viral attachment and entry. The envelope proteins of alphaviruses share conserved cysteine residues, which form structure-stabilizing disulfide bonds critical for maintaining the infective functions of the envelope spikes. Because disulfide bonds are formed almost exclusively through thioreductase reactions, alphaviruses very likely require host protein disulfide isomerase (PDI) for proper folding of the envelope proteins. Accordingly, we are attempting to develop host PDI as a novel drug target for treatment of alphavirus disease using CHIKV and current small molecule PDI inhibitors for a proof-of-concept. We have convincing evidence that PDI inhibitors potently inhibit CHIKV and VEEV replication *in vitro* with minimal cellular toxicity, not only indicating potential for drugability but also suggesting pan-alphavirus efficacy of future PDI inhibitors. Also under investigation is the effect of currently available PDI inhibitors on CHIKV genome replication and virion morphology, as well as the efficacy of these same inhibitors in relevant animal models of CHIKV. These data will establish a rationale for the development of new PDI inhibitors for the purpose of antiviral therapeutics.

189 Peptidomimetic inhibition of host-targeted serine proteases as a treatment against influenza

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The complications resulting from influenza virus infection are recognized as leading causes of hospitalization and deaths. Worldwide, epidemics are expected to cause severe illness in 3 to 5 million people and death in 250,000-500,000 infected patients. Preventive treatment is through vaccination and only neuraminidase inhibitors are currently recommended as first-line antiviral treatment. Mechanistically, proteolytic cleavage of the influenza virus surface glycoprotein hemagglutinin precursor (HA0) into HA1 and HA2 chains by host cell proteases is a necessary step for infectivity and virus spread. HA0 cleavage is required for virus-endosome membrane fusion and the subsequent release of the influenza virus genome into the cytoplasm. Recently, studies have shown that members of the mammalian type II transmembrane serine protease (TTSP) family are involved in HA0 proteolysis. For example, matriptase, TMPRSS2, TMPRSS4, DESC1 and HAT, which are present on the surface of airway epithelial cells, were found to correctly cleave HA0. Moreover, matriptase has the ability to process HA0 from the influenza virus H1 subtype including the known 2009 H1N1 pandemic virus. In this study, we developed and characterized a potent and selective peptidomimetic TTSP inhibitor (IN-1) based on the auto-activation sequence of matriptase onto which was added a C-terminal serine trap. We demonstrate the efficacy and selectivity of IN-1 *in vitro* and show that it diminishes virus replication of H1 and H3 subtypes in human epithelial respiratory cells (Calu-3). Moreover, IN-1 significantly reduces viral replication and morbidity in a mouse model without any noticeable toxicity on lung epithelium cells and tissues. Therefore, targeting host cell proteases using this class of inhibitors may well represent a promising and novel strategy to combat not only influenza but also other similar viral infections. This project is financially supported by NÉOMED.

190 Anti-influenza Virus Activity Mediated by Monoacetylcurcumin

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Curcumin (Cur) is a commonly used coloring agent and spice in food. Previously, we reported curcumin blocks influenza A virus (IAV) activity via multiple mechanisms. Structure-activity relationship analyses indicated that presence of the double bonds in the central seven-carbon chain enhanced the curcumin-dependent anti-IAV activity and curcumin might interfere with IAV entry by its interaction with the receptor binding site (RBS) of viral HA protein. Monoacetylcurcumin (MAC), a synthetic curcumin analogue, contains the critical structure involved in anti-IAV activity; however anti-viral activity of MAC has not yet been reported. In the present study, by comparing the effect of several structure analogues of curcumin on IAV infection, we found MAC reduced IAV infection to a similar extent to curcumin. As with curcumin, MAC attacked membrane structure in the liposome system, interfered with the activity of NA in MUNANA assay, and inhibited membrane fusion activity in hemolysis inhibition assay. While monitoring the cellular signaling pathways required for IAV replication we noticed MAC strongly inhibited phosphorylation (activation) of Akt and NF- κ B. However, unlike curcumin, MAC did not inhibit viral HA activity. Molecular docking results indicated that the replacement of acetyl group on one side of phenol ring lowered the fitness of MAC with RBS of HA protein. Taken together, MAC strongly inhibits IAV infection via similar mechanisms to curcumin, but it does not inhibit HA activity.

191 Exploring antiviral and Hsp90-inhibiting properties of marine alkaloid analogues through in silico modelling and in vitro screening

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Positive-stranded (+)-RNA viruses are dependent on host cell chaperones, which enable correct processing of viral proteins and thereby efficient virus replication.¹ Heat shock protein 90 (Hsp90) is a structurally conserved molecular chaperone with ATPase activity that facilitates viral replication by folding proteins involved in signal transduction, protein trafficking, receptor maturation and innate and adaptive immunity.²

Improved understanding of the host chaperone role in viral replication has raised interest in using Hsp90 inhibition as a strategy in antiviral drug discovery. This study, carried out in the context of the FP7 consortium MAREX³, encompassed *in silico* modelling to study the binding of synthetic marine alkaloid analogues to Hsp90. The studied compounds are analogues of alkaloids isolated from *Agelas* sponges. These alkaloids make interesting lead structures due to their various bioactive properties.

The antiviral potential of the compounds was evaluated in Hepatitis C (HCV) and Chikungunya virus (CHIKV) replicon cell models and the most promising CHIKV replicon inhibitors against infectious Semliki forest virus. Five compounds were identified as selective inhibitors of the HCV replicon with IC₅₀-values ranging from 8 to 24 µM. Two compounds dose-dependently inhibited the CHIKV replicon with IC₅₀-values of 42 and 67 µM, respectively. Molecular docking calculations showed that the ATP-binding site of human Hsp90 is indeed a putative target of the hits, and will be further assessed by evaluating the inhibition of Hsp90 ATPase activity *in vitro*.

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192 The Role of Pre-existing Antibodies in Determining the Efficacy of Vaccination in Humans

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BACKGROUND: Flaviviruses such as dengue virus (DENV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV) cause significant morbidity and mortality in endemic countries. The members of the genus are known to elicit cross-reactive antibodies which can be confounding in diseases like dengue fever where there is overwhelming evidence that severe disease is caused by the collaboration of pre-existing antibodies with DENV known as Antibody Dependent Enhancement. There is limited knowledge on how vaccine induced cross-reactive antibodies interact upon vaccination against different flaviviruses. Using the available safe vaccines against YF and JE we tested the hypothesis that cross-reactive antibodies impact antibody response to YF at the point of vaccination in a concentration-dependent manner by altering vaccine uptake and the innate immune response by antigen presenting cells. **METHODS:** Eighty-four (44 males) DENV IgG seronegative healthy volunteers, age 21-50 yr old (mean 32.1) were recruited and randomly assigned to four groups. Groups 1-3 received 2 doses of JE vaccine followed by YF vaccine at 1, 4 and 9 months post JE vaccination. Group four (control arm) received only YF vaccination. Blood samples were taken pre and post YF vaccination. JE and YF neutralizing antibody titers were measured and innate immune response characterized. **RESULTS:** We report the safety profile of sequential JE/YF vaccination and YF neutralizing antibody response at different time intervals post JE vaccination. **CONCLUSION:** YF vaccination within a short period after JE vaccination has safety profile comparable to YF vaccination in flavivirus naïve individuals.

193 **Selective Inhibitor of Nuclear Export (SINE) Compound, Verdinexor Alters New World Alphavirus Capsid Localization, and Reduces Viral Replication in Mammalian Cells.**

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Endemic to North, Central, and South America, the New World Alphaviruses cause a febrile illness that can progress to encephalitis with accompanying high morbidity and mortality rates in humans and equines. Three viruses in particular, Venezuelan, Western, and Eastern Equine Encephalitis Viruses (VEEV, WEEV, and EEEV), are of concern as naturally emerging infectious diseases and potential bioweapons. There are currently no drugs or vaccines approved for the treatment of alphaviruses in humans. Host targets offer the potential advantages of broad-spectrum activity across viral strains and lesser tendency for the development of viral resistance mechanisms. Exportin 1 (XPO1), which regulates nuclear export of >200 proteins, facilitates the nuclear export of alphavirus capsid protein, an activity essential for viral pathogenesis. We have developed potent, small molecule inhibitors of XPO1, termed Selective Inhibitor of Nuclear Export (SINE) compounds, with drugs from this class proving to be well tolerated and active in clinical studies of dog and human cancers. Treatment of cells with nuclear export inhibitors prior to infection significantly altered capsid subcellular localization compared to vehicle treated cells in a pattern similar to that seen following siRNA knock-down of XPO1. Quantitative PCR analysis revealed that extracellular viral RNA levels were reduced compared to intracellular RNA levels in cells treated with XPO1 inhibitors prior to infection. In plaque assays, cells treated with XPO1 inhibitors prior to infection with VEEV, EEEV, and WEEV showed significantly reduced viral titers compared to vehicle treated cells. Our experiments confirm the importance of host cell XPO1 nuclear transport protein in virus protein subcellular localization and replication of the New World Alphaviruses. These data suggest that inhibitors of the host cell protein XPO1 may prove useful as a therapeutic intervention by sequestering viral capsid protein in the nucleus and thereby disrupting viral assembly.

194 **Inhibition of cellular ER α -glucosidases as broad-spectrum strategy against acute virus infections**

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Inhibition of host functions essential for viral replication has been considered as an antiviral approach with potential to inhibit a broad spectrum of viruses as well as a high genetic barrier for the emergence of drug resistance. Especially, host-targeting broad-spectrum antivirals does not rely on time-consuming etiologic diagnosis and thus should be particularly valuable for management of viral hemorrhagic fever and respiratory tract viral infections, the medical conditions caused by many different enveloped RNA viruses and with a short time window for medical intervention. However, among a handful of cellular functions essential for viral replications, only the ER-resident α -glucosidases I and II have thus far been validated *in vivo* as the targets for broad-spectrum antiviral, mainly via disruption of glycoenvelop maturation. Specifically, previously we and others have shown that small molecular inhibitors of ER α -glucosidases I and II, *i.e.*, imino sugars, inhibit dengue, West Nile, Japanese Encephalitis, Marburg and Ebola viruses in mice. However, imino sugars are glucose mimetics that not only inhibit the ER, but also other host glucosidases, including gut maltase that breaks down ingested carbohydrates. Extensive chemical modification of N-linked alkyl side chain of DNJ derived imino sugars in our laboratory has yielded more than 200 homologs which resulted in some improvement in cellular uptake, but without drastic improvement of potency and specificity to the ER α -glucosidase enzymatic activities. In order to identify novel inhibitors that are potent and specific to the ER alpha-glucosidases, we have established cellular ER α -glucosidases I and II assays in 384-well high throughput format, using purified enzymes and a fluorogenic substrate. A pilot screening campaign with a small chemical library is currently under way. Hit molecules from such screening should have the potential to overcome the side effects of imino sugars, due to their off-target inhibition of other host glucosidases, as well as improve the antiviral efficacy of ER α -glucosidase-targeted antiviral therapy.

195 Orally available pyrazolopyrimidines effectively target a broad spectrum of enteroviruses by blocking capsid function

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Enteroviruses cause acute and chronic diseases such as common cold, meningitis, encephalitis, pneumonia, and myocarditis with or without consecutive dilated cardiomyopathy. The large number of enterovirus serotypes makes vaccine development difficult. Therefore, capsid-binding compounds with broad-spectrum anti-enteroviral activity e.g. pleconaril and vapendavir might be a good alternative for prevention and treatment enteroviral infections. But, safety and resistance concerns rose during experimental and clinical studies. Therefore, we searched for novel well tolerated, resistance-breaking compounds with distinct binding modes.

Here we report the structure-activity relationships of pyrazolopyrimidines, a well-tolerated and potent class of novel anti-enteroviral compounds. Inhibition of cytopathic effect of coxsackievirus B3, rhinoviruses 2, 8, 5, 42, and 48 by ~100 compounds was studied in HeLa cells. Highly active derivatives inhibit the replication of a broad spectrum of enteroviruses in vitro with lowest 50% inhibitory concentration between 0.04 and 0.64 μM against viruses resistant to pleconaril, used as control.

Using genetics and virological methods, viral capsid protein 1 was identified as a major target of the most promising, orally available compound, 3-(4-trifluoromethylphenyl)amino-6-phenylpyrazolo[3,4-d]pyrimidine-4-amine. Its binding to the viral capsid prevents initiation of the viral life cycle. The compound is well tolerated in mice and rats. Its prophylactic application dose-dependently prevented coxsackievirus B3-induced chronic myocarditis as well as lethal infection in mice. Moreover, its therapeutic effect was demonstrated with treatment begin on day 1 and 3 p.i. These promising results warrant further development of this compound as potential anti-enteroviral drug candidate.

196 Combination Therapy with Neuraminidase Inhibitor Oseltamivir and Polymerase Inhibitor T-705 Extends the Therapeutic Window against Highly Pathogenic Influenza H5N1 Virus

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The unusual severity of disease caused by highly pathogenic influenza H5N1 viruses, late identification of infected individuals, and development of drug-resistance represent challenges for successful treatment. To date, antiviral therapy of H5N1 influenza relies on neuraminidase inhibitors oseltamivir and zanamivir. We studied the effects of oseltamivir and T-705 (favipiravir, non-specific inhibitor of viral polymerases) used singly and in combination, where dosing started at 48, 72, 96 and 120 hours after infection of BALB/c mice with highly pathogenic A/Turkey/15/2006(H5N1) virus. In comparison to monotherapy, combination therapy with oseltamivir (20 mg/kg/d) and T-705 (50 mg/kg/d) resulted in 100% and 80% survival when dosed up to 96 and 120 hours after infection, respectively and reduced duration of illness at all tested time points. Reduced therapeutic efficacy was noted only in the group when treatment was initiated 120 hours after infection. Combination therapy reduced viral load in mouse lungs by four orders of magnitude compared to control animals ($P < 0.01$) and by two orders compared to monotherapy groups ($P < 0.01$). Immunohistochemical staining of H5N1 nucleoprotein demonstrated that combination therapy restricted the extent of virus spread within lung by preventing infection of neighboring cells. Histomorphometry revealed that combination treatment reduced the extent of lesions with active infection by 30% compared to oseltamivir and T-705 monotherapy. The high pulmonary expression of proinflammatory chemokines and cytokines in the control untreated animals was reduced in the group that received combination therapy, which contributed to the reduced pathogenicity of H5N1 infection and rapid recovery in mice receiving both drugs. Next generation sequencing showed that monotherapy and combination therapy affected the mutational landscape in the virus populations differently. Taken together, our findings indicate that combination therapy with oseltamivir and T-705 extended the therapeutic window against highly pathogenic H5N1 influenza virus infection in mice.

197 Analysis of serological and cellular immune correlates of protection against yellow fever infection induced by DNA-YFVax, a novel DNA-based live-attenuated yellow fever vaccine

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The yellow fever virus (YFV) causes life-threatening infections annually, despite the availability of a highly efficient live-attenuated vaccine [YFV-17D, Stamaril®], ~200,000 cases. Emergency vaccination is essential to control outbreaks, although the need for a proper cold-chain complicates the timely delivery of vaccines. Development of an easy and inexpensive DNA vaccine would allow a faster and simplified deployment. We developed a plasmid-based system (DNA-YFVax) that allows launching of recombinant YFV-17D *in vivo* directly from cloned cDNA and that protects against lethal YFV challenge in hamsters (Julander et al., ICAR 2015). We demonstrate that intraperitoneal and transdermal delivery of DNA-YFVax in mice induces virus-neutralizing antibodies with comparable kinetics and to equally high titers as Stamaril®. Neutralizing activity was detected as early as 7 days post-vaccination, which is mediated by IgM, in resemblance to humoral responses observed in humans immunized with Stamaril®. Flow cytometry analysis at day 5 post vaccination show that DNA-YFVax and Stamaril-inoculated hamsters had an increase in CD11c⁺, CD20⁺ and CD4⁺ cells in the spleen when compared to controls, indicating that dendritic cells (DCs), B and T CD4 lymphocytes were involved. Interestingly, DNA-YFVax induced higher numbers of DCs than Stamaril, which also expressed MHC II and CD40, indicating that DNA-YFVax may be even more effective than Stamaril® in stimulating protective adaptive immune responses. In conclusion, we show that DNA-YFVax is comparable to commercially available YFV vaccines regarding humoral and cellular immune correlates of protection. DNA-YFVax is a promising vaccine candidate and a viable alternative to current expensive and thermo-unstable YFV vaccines.

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198 Expression of HLA-DR on T lymphocytes in HIV-positive patients in dependence of HIV-1 tropism

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HLA-DR is one of immunity activation (IA) markers. Presently IA is key aspect of HIV pathogenesis and progression.

Aim of study was to establish expression of HLA-DR on T lymphocytes in HIV-positive patients in dependence of HIV-1 tropism.

Material and methods. Studied group included 29 HIV-positive patients who did not receive antiretroviral therapy (mean age – 33,5±7,2 yr., females – 16, males – 13). Clinical stages of HIV-infection were following: the 1st – 17 (58,6%) patients, the 2nd – 6 (20,7%), the 3rd – 5 (17,2%), the 4th – 1 (3,5%). The patients were divided in 2 groups according to HIV tropism: the group 1 – 19 patients infected by R5-tropic virus, the group 2 – 12 ones infected by non R5-tropic HIV. Detection of HIV tropism was performed by sequencing of V3 loop of gp120 gene («AmpliSens HIV-Resist-Seq», Russia). The cells immunophenotype has been established by using «FACSCalibur» flow cytometer («Becton Dickinson», USA).

Results. In patients of the 1st group the higher expression of HLA-DR on T lymphocytes, cytotoxic T lymphocytes and T helpers was detected in comparison with patients in the 2nd group (median (min – max)): 713,49 (206,1 – 1626,5) v. 427,67 (143,0 – 1023,8), p< 0,05; 764,52 (61,6 – 1655,3) v. 417,30 (152,3 – 952,4), p< 0,03; 70,03 (18,4 – 163,6) v. 54,44 (24,1 – 153,5), p< 0,06, respectively, Mann-Whitney test.

Conclusions. Infection with R5-tropic HIV is associated with more expressed T cell mediated immunity activation. It may propose the additional indications for CCR5 coreceptors antagonists administration.

199 New Antiviral Properties of 5'-Norcarbocyclic Nucleoside Analogues

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Modified nucleosides have provided a wealth of drug candidates and clinically approved therapeutics. The replacement of the furanose oxygen in the sugar moiety with a methylene group, yielding a class of nucleosides, known as carbocyclic nucleosides. This modification increases stability and lipophilicity to the analogues. Potent antivirals such as Lobucavir, Abacavir and Entecavir are important members of carbocyclic nucleosides in the field of medicinal chemistry.

Recently we found new interesting biological properties of 5'-norcarbocyclic nucleoside analogues. A series of 1-(4'-hydroxy-2'-cyclopenten-1'-yl)uracil derivatives with long alkynyl substituents completely inhibited the growth of *M. tuberculosis* culture at a concentration of 5-20 µg/mL. Analogues of 2',3'-dideoxy-2',3'-didehydro-5'-noruridine with three different types of modifications showed inhibitory activity against HIV-RT wild-type (IC₅₀ 5-10 µM) and mutants L100I (IC₅₀ 1.2-2.1 µM) and K103N (IC₅₀ 8-17 µM).

In an attempt to further explore and optimize therapeutic potential of carbocyclic uridine derivatives, different aspects of the scaffold were investigated. Based on the activity exhibited by the C5 substituted pyrimidines (such as brivudine (BVDU) and its carbocyclic version (C-BVDU), a series of 5-arylamino-uracil derivatives of 2',3'-dideoxy-2',3'-didehydro-5'-noruridine were synthesized. In that regard, 5-phenylamino-uracils were condensed with epoxy-cyclopentene in the presence of a palladium catalyst using Trost methodology. The compounds then were tested against RT HIV-1. Disappointingly, only weak activity was found (K_i 60-100 µM).

In order to optimize low solubility and inhibitory properties of 5'-norcarbocyclic analogues of 2',3'-dideoxy-2',3'-didehydro-uridine, a group of phosphonates was designed and synthesized. Additional studies to explore anti-HIV properties of such phosphonates will be pursued.

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200 Early Inhibition of Human Cytomegalovirus Replication as a Therapeutic Option: Design, Identification, and Characterization of New Anti-Hcmv Candidate Drugs With a Novel Mechanism of Action

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Human cytomegalovirus (HCMV) is a major opportunistic pathogen in immunocompromised individuals and also plays an important role in chronic inflammatory diseases. In addition, it is the leading viral cause of congenital birth defects. To date, no vaccine is available for the prevention of HCMV infections and for the treatment of congenital infection. Moreover, only a few drugs are licensed to manage HCMV diseases, whose clinical utility is often limited by several drawbacks. Thus, there is still a strong need to develop new, safe, and effective antivirals with a new mechanism of action. The identification of the viral and/or cellular factors regulating the very early virus-host cell interactions as well as the characterization of the first viral proteins expressed in infected cells may provide the rationale for the design of alternative antiviral strategies. We focused our recent activity on the identification and characterization of new early-acting anti-HCMV compounds. We identified some sulfated derivatives of *E. coli* K5 capsular polysaccharide as inhibitors of HCMV attachment and entry. These compounds are able to block HCMV attachment and entry by competing with the binding of viral glycoproteins to cellular heparan sulfate proteoglycans. Furthermore, we describe a 6-aminoquinolone compound -WC5- and some analogs endowed with potent anti-CMV activity and that target the essential Immediate-Early 2 protein by blocking its binding to crucial viral responsive promoters. Importantly, this mechanism seems to be conserved in MCMV, thus paving the way to further preclinical evaluation in an animal model. Finally, we describe the design, set up, and preliminary results of a screen of a small molecule library aimed at identifying early inhibitors of HCMV that act prior to DNA synthesis.

201 **Anti-tumor effect of cidofovir against human papillomavirus positive and negative cells is not exclusively due to DNA damage.**

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INTRODUCTION: Human papillomavirus (HPV) can induce both benign and malignant hyper proliferations. It is involved in almost all cervix carcinoma and in an increasing amount of head and neck cancers. Cidofovir (CDV) was found to be efficacious in the treatment of HPV related lesions. In this study, we evaluated whether a correlation exists between DNA incorporation, DNA damage and the anti-proliferative effects caused by CDV. We will provide a better insight on how CDV kills specifically tumor cells, without harming normal cells.

METHODS: In the present work, HPV+ and HPV- cells of both cervix carcinoma and head and neck squamous cell carcinoma were included. As a control, we compared with primary human keratinocytes (PHKs), human embryonic lung (HEL) fibroblasts and primary epithelial tonsil (PET) cells. The anti-proliferative effect is expressed as the concentration needed to inhibit cell growth by 50% (CC₅₀). CDV drug metabolism and incorporation into genomic DNA was studied by use of radiolabeled CDV and metabolites were separated by HPLC. DNA damage was evaluated by a flow cytometry assay using a double staining with propidium iodide and a monoclonal antibody against -H2AX, a selective marker for double stranded DNA breaks.

RESULTS: Our results showed that the levels of intracellular CDV metabolites were higher in PHKs and PET cells compared to HPV+ and HPV- tumor cells and HEL cells. However, the amount of CDV incorporated in tumor cells was higher compared to normal cells. We demonstrated that CDV causes double stranded DNA breaks in each phase of the cell cycle. The percentage of cells that are -H2AX positive was higher in the tumor cells compared to the normal cells. CC₅₀ values, CDV incorporation and DNA damage were compared to investigate whether a correlation exists between them. We observed a significant correlation between DNA incorporation and DNA damage and between CDV incorporation and CC₅₀. No correlation was observed between CC₅₀ and DNA damage.

CONCLUSIONS: Taken together these results indicate that the CC₅₀ value results from incorporation of CDV into genomic DNA which causes DNA damage. However the anti-tumor effect of CDV cannot be explained exclusively by DNA damage.

202 **The homodimerization of Human Papillomavirus oncoprotein E6 as a target for the development of new anti-HPV drugs.**

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High-risk human papillomaviruses (HPV) are the most common cause of cervical cancer and their association with some forms of head and neck cancer has recently emerged. Among the variety of high-risk genotypes, HPV16 and HPV18 are the main aetiological agents. During viral infection, two oncoproteins, E6 and E7, are expressed from the early phases of viral life cycle and their expression drastically increase after the integration of viral DNA into host genome. E6 is an essential factor to induce proteasomal degradation of many cellular proteins important for apoptosis, cell adhesion, and cell cycle control such as p53, Scribble, and p300 respectively. Recent *in vitro* evidences highlighted the possible existence of E6 as a functional dimer, suggesting the importance of the dimerization for the successful degradation of p53. However, no demonstration of E6 dimerization in living cells has been given yet. On this basis, we focused on the study of the intracellular homodimerization of HPV16 E6 by using two cell-based assays to detect the E6 dimeric form, with the goal to develop new drugs able to interfere with its self-association process. We used Bimolecular Fluorescence Complementation (BiFC) technique and successfully visualized a green fluorescent signal of complementation in C33A cells. In addition, we employed a Bioluminescence Resonance Energy Transfer (BRET) assay and quantitatively detected the self-association of the N-terminal domains (E6N) of the viral protein in HEK 293T cells. In the next future, we plan to study the importance of the dimerization for the degradation activities of E6 towards three selected targets: p53, Scribble, and MAGI-1. Finally, an *in silico* screening of a small-molecule library has been performed using the NMR structure of E6N as a template and ten compounds have been selected to be tested for inhibition of E6 dimerization in an ELISA-based *in vitro* assay.

203 Over Additive Effect of Combination of Oseltamivir and Vitamin E on Oxidative Damages in Liver Caused by Influenza Virus Infection in Mice

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Influenza virus infection has established itself as a significant threat to human health, work disability and often severe health complications, i. g. oxidative stress and liver disorders, and most effective therapeutic strategy is still need. The drugs selected for the study have different mode of action. Vitamin E belongs to the class of essential antioxidants, and has been long known for its protection of tissues during inflammatory illnesses. Oseltamivir is neuraminidase inhibitor with a highly specific action against influenza virus type A and B. This work aims to investigate the effect of combinations of oseltamivir and vitamin E on oxidative damage in the liver of mice experimentally infected with influenza virus and to select the lowest effective doses of these combinations.

Male mice were inoculated intranasally with influenza virus A/Aichi/2/68/(H3N2) (10 of MLD₅₀). Vitamin E was applied intraperitoneally for five days before viral challenge in single daily dose of 120 mg/kg b.w. Oseltamivir was administered *per os* in five days course beginning 4 hours before viral inoculation in a doses of 2.5, 1.25 and 0.62 mg/kg b.w. Markers of oxidative damages (endogenous glutathione malondialdehyde, activities of glutathione peroxidase, superoxide dismutase, catalase) were measured on the 5th day after virus inoculation.

We found that the most successful drug combination for liver prevention against oxidative damages is application of vitamin E for five days before viral inoculation and administration of oseltamivir in a dose of 1.25 mg/kg b.w.

204 Spectrum of Activity Testing for Dengue Therapeutics in AG129 Mice; Proof-of Concept Studies With NITD-008

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Dengue is arboviral disease caused by 4 related flaviviruses (DENV-1 to DENV-4). The development of therapeutics is hampered by the paucity of good preclinical disease models. Interferon α , β , γ receptor deficient AG129 mouse models of DENV-2 disease have been an important advance. However, comparable models of DENV-1, DENV-3 and DENV-4 disease are needed. We recently developed such models for DENV-3 and DENV-4 using low-passage non-mouse adapted viruses which produce rapid disseminated disease with high lethality. Here, we compared the protection afforded by treatment with the adenosine nucleoside NITD-008 in these models. Groups of AG129 mice (n=24) were inoculated with 7.0 Log₁₀ plaque or focus forming units of DENV-2, DENV-3 and DENV-4. Twelve animals from each group were treated orally with NITD-008 (20mg/kg) twice daily for 4 days beginning immediately after inoculation. Remaining animals were vehicle treated controls. All vehicle treated mice experienced high titer viremia on days 2 and 3 post inoculation (pi.). All NITD-008 treated mice also developed viremia. In DENV-2 and DENV-3 infected treated animals, viremia levels were comparable to controls on day 2 pi. but, significantly lower on day 3 (p=0.0013 DENV-2; p=0.0006 DENV-3) while in DENV-4 animals treatment significantly reduced viremia on both days 2 (p=0.006) and 3 pi. (p=0.015). The course of disease was followed to day 28 days pi. Vehicle treated mice infected with each DENV developed rapid progressive disease with high lethality. NITD-008 treatment altered disease course for each DENV, although the impact varied. For DENV-2, treatment significantly increased survival (p=0.014) and extended the mean day of death (MDD) in animals that died (p= 0.002). For DENV-3-infected mice, survival was also increased with no lethality seen (p< 0.0001). In contrast, despite reducing viremia in DENV-4 challenged animals, NITD-008 treatment failed to increase survival although, the MDD was extended (p< 0.0001). These studies demonstrate that early-stage in vivo spectrum of activity testing of therapeutic candidates is feasible and is an important tool to help prioritize the best candidates for further development.

205 Limited evolution and (almost) clonal expansion of the yellow fever virus during lethal infection in mice, implication for vaccine and drug development against flaviviruses

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Very little is known about the intra-host diversity and evolution of the yellow fever virus (YFV) and other flaviviruses. In analogy to other RNA viruses that use an error-prone replication strategy (such as the hepatitis C virus), substantial diversification can be expected during replication in the vertebrate host. By infecting AG129 mice (that have a defect in innate immunity) with a YFV-17D vaccine strain (Stamaril®), we assessed the dose dependence and the evolutionary consequences of neurotropic yellow fever infection *in vivo*. Infection with an inoculum ranging between 10^5 and 1 PFU resulted rapidly and uniformly in virus induced morbidity and mortality [mean day of euthanasia (MDE) 12 ± 2 days p.i.]. Even an inoculum as low as 0.01 pfu resulted in disease and mortality (MDE 17 ± 2), lower inocula did not cause disease but occasionally induced seroconversion. Subsequently, viruses isolated from mouse brains were plaque purified (comparable plaque morphologies were noted); about ten plaque-purified virus clones were sequenced per mouse brain and were compared to the inoculated virus. Overall, the brain derived YFV-17D RNA populations (resulting from inoculation of 10^5 PFU of Stamaril®) were very homogenous, with error-rates ranging from 1–8 changes per clone and Shannon entropies of 0.002–0.01. Next mice were inoculated with DNA-YFVax, a novel plasmid-based system that allows the direct launching of clonal YFV-17D *in vivo*. YFV-17D derived from mice infected with this DNA-YFVax reached error rates and Shannon entropies of zero. Together this demonstrates that the heterogeneity in Stamaril® infected mice is the likely consequence of pre-existing heterogeneity in the current vaccine Stamaril®. Thus, we demonstrate that (in contrast to many other RNA viruses) YFV-17D (and likely also other flaviviruses) do not necessarily evolve to highly diverse viral quasi-species during replication in the vertebrate hosts. In view of vaccine and antiviral drug development, this characteristic may largely lower the risks of antigenic drift and development of antiviral resistance, respectively.

206 Identification of novel cellular targets by screening a library of known bioactive molecules and their potential use in anti-HIV therapeutic regimen

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Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immuno-deficiency syndrome (AIDS) that has created a global pandemic for more than three decades with major epicenters currently being Africa and South-east Asia. HIV-1 efficiently hijacks the host cellular machinery not only to complete its life cycle but also to escape the human immune defense system. The current strategy for the management of HIV infection is Highly Active Antiretroviral Therapy (HAART), a therapeutic regimen comprising of a combination of viral inhibitors. With an arsenal of more than twenty five FDA approved molecules against HIV, we have succeeded to control or manage AIDS to a significant extent but have failed to cure or eradicate the disease. Apart from the emergence of drug resistant viral strains and drug toxicity to host, the major drawback of the present therapeutic regimen is its inability to identify and destroy the latent viral reservoirs. Thus HAART is not the ultimate answer for AIDS patients and there is a need to identify novel therapeutic strategies. All the events of HIV lifecycle, including latency and cell-cell transmission, are predominantly regulated by a large number of cellular factors. Hence, targeting cellular factors in association with viral factors may give us an advantage in eliminating the virus from the host. The objective of the present study was identification of such cellular targets, inhibition of which will not affect the host cell but at the same time will efficiently inhibit virus infection and replication. To achieve this aim, we have screened a library of more than a thousand pharmacologically active compounds, with known cellular targets and mechanism of action, for their potential as anti-HIV molecules using reporter cell system. Several of these molecules have shown significant anti-HIV activity at nano-molar or low micro-molar concentration that has been further validated in a T-cell line. The screening results also indicate that many of the currently used therapeutic molecules for other diseases have significant anti-HIV activity. Our results till date indicate that few of these molecules inhibit HIV-1 at early stage of infection by modulating cellular pathways.

207 **N-Methanocarbothymidine is Effective Against HSV-1 and VZV in Mice**

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N-Methanocarbothymidine (N-MCT) is known to prevent herpes simplex type 2 (HSV-2) infections in mice and guinea pigs. We evaluated N-MCT in mouse models of HSV-1 and varicella-zoster virus (VZV) using oral and topical routes, and the compound was highly potent in all assays. SKH-1 mice were infected with HSV-1 by scarifying the snout, and were then treated topically with vehicle, Zovirax or N-MCT in carbopol gel. Treatment began 1 dpi and was applied to the site of inoculation three times daily at 6 h intervals through Day 7. All mice in the vehicle group died. N-MCT prevented mortality at all doses (0.05, 0.5 or 5%), as did Zovirax, the positive control. N-MCT and Zovirax also eliminated lesions in all mice, so that lesion scores were significantly reduced ($p < 0.001$) compared to the vehicle group. The site of HSV-1 infection was swabbed and virus was titered. N-MCT and Zovirax treatments caused significant reductions in mean peak virus titer ($p < 0.01$) compared to vehicle. SCID-hu mice with human skin xenografts were infected intradermally with VZV and treated orally twice daily with vehicle, foscarnet (positive control), or N-MCT at 5, 10, 20, 25, 50, or 100 mg/kg/day. Virus spread was measured daily by in vivo bioluminescence imaging (IVIS). All doses of N-MCT and foscarnet significantly reduced VZV yield and growth rates ($p < 0.0001$) compared to vehicle. A skin organ culture model was used to evaluate N-MCT as a topical treatment for VZV. Human skin explants were inoculated with VZV by scarification and treated daily from 1-4 dpi with N-MCT (0.05, 0.5, or 5%) or cidofovir (0.5%, positive control) in a carbopol gel. VZV yield was measured by IVIS on day 5. All doses of N-MCT and cidofovir significantly reduced VZV yield ($p < 0.0004$) compared to vehicle. Studies are currently underway to determine the effects of delaying topical N-MCT treatment to 3-6 or 5-8 dpi on VZV yield in skin organ culture. N-MCT is a potent and promising drug for treating cutaneous herpesvirus infections.

208 **Inhibition of Hepatitis C Virus in Mouse Models by Lipidoid Nanoparticle-mediated Systemic Delivery of siRNA against PRK2**

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Host factors play a crucial role in hepatitis C virus (HCV) infection and propagation and are promising targets for anti-HCV therapy development. However, the therapeutic potential of small interfering RNAs (siRNAs) targeting proviral host factors remains largely unexplored. We designed siRNAs targeted against protein kinase C-related kinase 2 (PRK2), which phosphorylates HCV RNA polymerase and promotes HCV replication. Isotype-specific silencing of PRK2 with siRNAs, but not PRK1 or PRK3, resulted in HCV replication suppression. Anti-HCV efficacy of PRK2-targeting, chemically modified siRNAs was evaluated using immunodeficient mice implanted with a human hepatocellular carcinoma Huh7 cell line-derivative that constitutively produces infectious HCV particles as a model. Systemic delivery of siRNA using lipidoid nanoparticles (LNPs) at a dose of 1 mg/kg body weight resulted in 70% PRK2 expression reduction in hepatocytes. Three injections of the siRNA LNP, administered every three days at a dose of 3 mg/kg, displayed potent anti-HCV efficacy. Subcutaneous and orthotopic xenograft mouse models showed a 3.72 and a 1.96 log₁₀ reduction in serum HCV RNA level, respectively. Our results demonstrate for the first time the in vivo role of PRK2 in HCV replication and that a host factor-targeting, siRNA-based approach is a potentially promising HCV infection treatment.

209 **Human beta-defensins 2 and 3 bind and inactivate intracellular human immunodeficiency virus in oral epithelial cells**

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Human beta defensin 2 (hBD2) and 3 are cysteine-rich cationic innate proteins with anti-human immunodeficiency viral (HIV) properties. Although the anti-HIV activity of hBD2 and hBD3 has been extensively investigated in HIV-susceptible CD4⁺ T lymphocytes, the mechanisms of antiviral functions of defensins in mucosal epithelial cells, the first target for viral transmission, have been poorly understood. We previously showed that high-level expression of hBD2 and hBD3 in polarized adult oral epithelial cells inactivates HIV and reduces transcytosis of infectious virions. Here we investigated the molecular mechanisms of hBD-mediated HIV inactivation in polarized epithelial cells. We found that heparan sulfate proteoglycans (HSPG) on the apical surfaces of polarized cells facilitate the binding of hBD2 and hBD3 to the cell surface and to the envelope of dual-tropic, X4- and R5-tropic HIV-1 viruses. Binding of hBDs to HIV on the cell surface did not affect viral attachment and infectivity of cell-bound virions. Virus-bound hBD2 and hBD3 were cointernalized with virions into endosomes, formed oligomers, and inactivated virus in the vesicles. The anti-HIV effect of combined hBD2 and hBD3 was substantially higher than that of the individual proteins. In a cell-free system

without HSPG, the hBDs did not bind to the HIV envelope and did not inactivate HIV. These results reveal a mechanism of protection of adult oral epithelium from HIV transmission; i.e., oligomer forms of hBD2 and hBD3 inactivate virus in the vesicular/endosomal compartments of oral epithelial cells. Newborn/Infant oral epithelia lack hBD2 and hBD3 expression, and approaches designed to induce their expression in infant oral epithelium or the topical application of recombinant hBD2 and hBD3 could prevent postnatal HIV mother to child transmission. Such strategies could also be useful for preventing HIV transmigration through other mucosal sites, including the vaginal and cervical epithelia in human adults.

210 **Druggable Protein-Protein Interactions as Antiviral Targets for an Innovative Chemotherapeutic Intervention**

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Most cellular and viral processes depend on the coordinated formation of protein-protein interactions. A novel strategy to inhibit virus replication is based on the disruption of viral protein-protein complexes by molecules that mimic either part of the subunits interaction. In the past few years, our research interest has been focused on the development of new anti-herpesvirus inhibitors which act by disrupting the interactions between viral enzyme subunits, including those of HSV-1 ribonucleotide reductase, and HSV-1 and HCMV DNA polymerase. As an example, in a high-throughput screening of small molecules libraries we identified some compounds that are able both to specifically interfere with the interactions between the two subunits, UL54 and UL44, of HCMV DNA polymerase and to block virus replication in HCMV-infected cells. More recently, we focused our interest on influenza virus RNA polymerase, which is a complex consisting of three proteins (PB1, PB2, and PA). Taking advantage of a crystallographic structure of a truncated form of PA bound to a PB1-derived peptide, we performed an *in silico* screening of 3 million small-molecule structures to search for inhibitors of the PA-PB1 interaction. From this screening, several compounds emerged that are able to interfere with the interaction between PB1 and PA both *in vitro* and in cells. Some of these molecules also inhibited the replication of a panel of influenza A and B viruses, including an oseltamivir-resistant isolate, with EC50 values in the low micromolar range. Importantly, preliminary studies indicate that these small molecules also exhibit considerably lower propensity to develop drug resistance than other anti-influenza compounds, e.g. oseltamivir and rimantadine. Finally, recent studies suggested the importance of the dimerization of human papillomavirus oncoprotein E6 for successful degradation of p53. Thus, we are currently working on the development of new anti-HPV drugs able to interfere with the E6 dimerization process.

211 **Phosphonate Prodrugs of Nucleoside HIV RT Inhibitors**

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Despite the numerous drawbacks, 3'-azido-3'-deoxythymidine (AZT, Zidovudin, Retrovir) remains one of the key drugs used in the treatment and prevention of HIV infection in both monotherapy and HAART. A strategy in searching for new effective and safe AZT agents among latent (depot) forms of AZT has yielded its positive results. In particular, AZT 5'-H-phosphonate (Nikavir[®], phosphazide) has demonstrated clinical advantages over parent AZT: first and foremost, lower toxicity and better tolerability. It can be effectively used for the prevention of vertical transmission from mothers to babies and as an alternative drug for HIV-infected patients with low intolerance to Zidovudin [1]. Preclinical studies of another phosphonate, AZT 5'-aminocarbonylphosphonate, have demonstrated that it releases AZT when taken orally. Pharmacokinetic studies have shown a prolonged action potential [2]. Preliminary results of clinical trials are promising: AZT 5'-aminocarbonylphosphonate is nontoxic for patients at single and multiple oral doses up to 2000 mg (stage I) and effective in monotherapy at multiple oral doses 1200 mg taken once a day (stage II).

O-(L-2',3'-Dideoxy-3'-thiacytidine-5'-yl)-O'-(3'-azido-3'-deoxythymidine-5'-yl)aminocarbonylphosphonate can be regarded as a depot form of both Retrovir and Lamivudine, the drugs composing the anti-HIV cocktail Combivir[®]. The compound showed high anti-HIV activity both *in vitro* (in cell cultures infected by HIV-1) and *ex vivo* (human tonsil tissues infected by HIV-1) [3]. This phosphonate is stable in acid media and is rather rapidly hydrolyzed under neutral and alkaline conditions to give not only the parent nucleosides but also the corresponding 5'-aminocarbonylphosphonates which are known as depot forms of AZT[4] and 3TC[5].

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212 Regulation of HPV16/18 E6, p53 and ATM in parental and CDV-resistant cells after cidofovir treatment.

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INTRODUCTION: Cidofovir (CDV) displays potent activity against a broad spectrum of DNA viruses and it is effective in treatment of human papillomaviruses (HPVs)-induced neoplasias. Recently, whole-genome expression profiling and bioinformatics analysis using IPA software showed that p53 and ATM pathways were differently affected by CDV treatment in primary human keratinocytes (PHKs), immortalized keratinocytes (HaCaT), HPV16⁺ (SiHa) and HPV18⁺ (HeLa) cervical carcinoma cells. In addition, these pathways seemed to be altered in cells that have been selected for resistance to CDV.

METHODS: We evaluated the expression of HPV16/18 E6 and p53, as well as expression and phosphorylation of ATM by Western blot. Total protein extract of PHKs, HaCaT, SiHa, HeLa and their CDV-resistant counterparts (HaCaT_{CDV}, SiHa_{CDV} and HeLa_{CDV}) were obtained after 1, 3, 5 and 7 days treatment with 50 µg/ml CDV.

Results: CDV treatment decreases the expression of E6 in SiHa cells (26% at day 3 and 63% at day 7) and in HeLa (about 70% at days 5 and 7). This effect is abrogated in SiHa_{CDV} and HeLa_{CDV} cells.

Constitutive p53 expression is stable in PHKs over time and treatment with CDV increases it at days 1, 3 and 5. On the other hand, constitutive p53 expression declines over time in HeLa and SiHa cells and CDV treatment prevents this decrease. In contrast, p53 expression does not seem to be modulated by CDV in HaCaT cells, which express elevated levels of p53. While p53 amounts are almost undetectable in HaCaT_{CDV} and SiHa_{CDV}, p53 expression is higher in HeLa_{CDV} compared to parental HeLa. Total ATM expression appeared to be modulated only in treated-SiHa parental cells at day 7. However, activity of ATM (p-ATM) is increased in PHKs, HaCaT, HeLa and SiHa after CDV treatment. Activity of ATM is also increased in HaCaT_{CDV} and SiHa_{CDV} after CDV treatment, but not in HeLa_{CDV}.

CONCLUSION: Taken together, this data showed that CDV treatment decreases the expression of E6 in HeLa and SiHa, and induces ATM activation in almost all the cell lines (except in HeLa_{CDV}). Our next purpose is to investigate whether E6 and ATM modulate p53 in parental cells but most importantly in resistant cells.

213 Targeting Intracellular Potassium for the Control of Respiratory Syncytial Virus

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Respiratory syncytial virus (RSV) is the most important viral agent of pediatric lower respiratory tract illness worldwide. Despite the disease burden presented by RSV, no vaccine is available and treatment remains non-specific. In the absence of effective and economical treatments, new drug candidates are needed to combat RSV. Towards this goal, we have successfully designed a high-throughput screen to identify inhibitors of the RSV life cycle. We identified 67 'high potential' compounds that significantly reduced RSV infectivity. Strikingly, ~30% of the discovered compounds are known to modulate intracellular potassium ([K⁺]_i) levels. From these, we selected the cardiac glycoside, digoxin, and the potassium ionophore, valinomycin for further analysis in HEp-2 cells. Both digoxin and valinomycin reduced RSV infection by >95% (IC₅₀: 0.016 µM and 0.00024 µM respectively) without affecting cell viability at concentrations well above IC₅₀ (CC₅₀: 1.6 µM and 1.2 µM respectively). Additionally, both digoxin and valinomycin resulted in a dose-dependent reduction in [K⁺]_i as assessed by a fluorescence indicator dye (APG-4). Culturing cells in low potassium media has also been shown to reduce [K⁺]_i. RSV infected HEp-2 cells cultured in potassium lacking media (0 mM KCl) for 48 hours resulted in reduced RSV replication, suggesting any observed antiviral effects are potassium dependent. To further refine where in the RSV replication cycle [K⁺]_i depletion exerts an antiviral effect, we utilized a time of addition assay in HEp-2 cells. Addition of digoxin or valinomycin as late as 8 hours post infection significantly inhibited RSV replication. Finally, to better model the *in vivo* environment, we infected primary human nasal epithelial cells grown in an air liquid interface and treated with digoxin. Cells treated with digoxin had reduced RSV in the airway surface liquid. Further studies are ongoing to elucidate the mechanism driving RSV dependence on [K⁺]_i.

214 DNA Polymerase Inhibitors Enhance the Anti-Viral Effect of Terminase Inhibitors when used in Combination against HCMV

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Human cytomegalovirus (HCMV) infects roughly 80% of the world's population and can prove detrimental in immunocompromised and immunologically immature individuals. Complications that arise from a HCMV infection include retinitis (AIDS patients) and mental retardation (neonates). All currently approved pharmacotherapies – ganciclovir (GCV), its oral prodrug valganciclovir, cidofovir (CDV), and foscarnet– elicit an anti-viral effect by targeting the viral DNA polymerase. Since recurrence of infection can occur upon cessation of therapy, lifelong adherence is necessary. Therefore, HCMV strains with decreased susceptibility to drug are common (drug resistance). Additionally, because all currently approved therapies share the same viral target, the incidence of cross-resistance is high. The benzimidazole ribonucleosides (BDCRB) and deoxyribosylindole nucleosides (Indole 1896) exert their effects late in the viral replication cycle by targeting the HCMV terminase, the enzyme responsible for cleaving and packaging viral DNA into virions. Since these compounds exhibit a unique anti-viral mechanism but along the same replication pathway as current therapies, there is a possibility for a positive pharmacodynamic drug interaction. Therefore, we hypothesize that the combination of DNA polymerase and terminase inhibitors will result in a synergistic anti-viral effect. Combination viral plaque reduction assays, with a 95% confidence interval, demonstrated high synergy indexes: 89.0 (GCV and BDCRB), 74.2 (GCV and Indole 1896), and 79.3 (BDCRB and CDV). These results indicate a highly synergistic relationship between the anti-viral DNA polymerase and terminase inhibitors. We therefore conclude that the combination of HCMV DNA polymerase and terminase inhibitors can delay the onset of viral resistance, lower the incidence of cross-resistance, and enhance the combined anti-viral efficacy.

215 Anti-HSV activity of *Pedilanthus tithymaloides*, an Indian folklore, through the inhibition of toll-like receptor 3 signaling pathway

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In our continued quest for validating the ethnomedicinal practices of different tribes of India we have isolated the flavonoid luteolin from the folklore *Pedilanthus tithymaloides* with antiviral activity. The MTT and plaque reduction assay showed that both the extract and the isolated luteolin had potent antiviral activity against wild-type and clinical isolates of HSV-1 (EC₉₉ 78.4 and 29.4 µg/ml). The inhibitory effect was significant when the extract or compound were added at 2 h prior to, during or 4 h post-infection. The addition of luteolin at or during or 4 h post-infection showed a similar inhibitory effect, indicating that the mode of action of luteolin is not only the viral entry but also in the post-entry events. Earlier studies have reported NF-κB activation for effective replication of HSV and its suppression to inhibit HSV infection. We have demonstrated that the extract or luteolin suppress the NF-κB activation during HSV-1 infection.

The *in vivo* studies revealed that the extract or luteolin at its virucidal concentration was nontoxic and reduced virus yield in the brain of HSV-1 infected mice in a concentration dependent manner, compared to skin tissues. Furthermore, both extract and luteolin significantly reduced *Tumor necrosis factors* (TNF)-, Interleukin (IL)-1, IL-6, and gamma interferon (IFN-) expression, related to TLR3 signaling. Interestingly, following HSV infection luteolin treatment decreased the TLR3 mRNA expression but inhibited the upregulation of TRIF and NF-κB expression. These results collectively suggested that the extract or luteolin treatment altered HSV induced inflammation via a TLR3 signaling pathway.

216 Efficacy of Alpha – Zam in The Therapy of HIV Infection Among Individuals Seeking Alternative Therapy in Nigeria

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The prevailing limited access to antiretroviral therapy and the current incurability of HIV/AIDS explains the continued and potentially increasing use of herbal remedy among AIDS patients. In Nigeria, herbal therapists claim dramatic results when treating patients with HIV infection and even full-blown AIDS, but the evidence to support their claims is anecdotal or patchy and therefore would require investigations to demonstrate the therapy's effectiveness. Therefore this study was designed to investigate the anti-retroviral activity of alpha-zam (a-zam) using participant's sera for virological and immunological assays. Fifty one patients at

different stages of HIV infection and seeking treatment at a herbal centre were recruited for this study. Participants took 10mL of a-zam reconstituted in 50mL of warm water. This was done three times daily for a period of 3 months. Patient's sera were assessed at baseline and at the end of the study for CD4 counts and HIV RNA viral load. Signs and symptoms associated with HIV infection among participants fully subsided within a month of taking the herbal concoction and there was a significant rise in CD4 count ($p < 0.05$). This increased by an average of 262, 310, 456 and 510 cells/ μ l in patients at stages I to IV of HIV infection respectively. Forty-one (80.4%) patients had undetectable viraemia at the end of the therapy while the remaining 10 (19.6%) at stage IV had a marked reduction from an average of 51000 copies/ml to < 1000 copies/ml. It is concluded that a-zam is an effective anti-HIV agent causing significant increase in CD4 counts and marked decrease in viral load. Work is on-going to establish the anti-retroviral activity of a-zam using various in-vitro assays.

217 A G-quadruplex Forming Aptamer Potently Inhibits HIV-1 Entry into the Host Cell

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G-rich aptamers that form stable G-quadruplex structures have been reported to display increased cell uptake, low degradation rates and promising anti-HIV-1 activity mainly by acting as integrase inhibitors. Here we investigated the antiviral activity of a G-quadruplex aptamer that has been reported to displays antineoplastic properties both *in vitro* and *in vivo*. The antiviral activity of the G-quadruplex aptamer has been investigated towards different HIV-1 strains, host cells and times post-infection. Fully susceptible HIV-1 strains as well as strains resistant to current antiretroviral drugs were considered. Both actively and persistently infected cell were assayed; PBMCs from healthy donors and HIV-infected patients were also tested. We demonstrated that the G-quadruplex aptamer was highly active as HIV-1 inhibitor acting within 1 hour post-infection, which corresponds to the stage of viral attachment/entry. The aptamer displayed IC₅₀ values in the low nanomolar range, while cytotoxicity was in the high micromolar range, hence with extremely promising selectivity indexes. These findings strongly indicate the use of this G-quadruplex aptamer as a new potent and safe anti-HIV-1 agent.

218 AR-12 – A Novel Broad Spectrum Host Directed Antiviral Drug

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AR-12 has been identified as the lead compound of a new class of potential antiviral agents which do not target viruses directly, but function by interfering with viral replication dependent upon host cell activities. Targeting the host cell activity can potentially result in broad-spectrum antiviral activity.

AR-12 primary mechanism of action is the inhibition of GRP78 (BiP, HSPA5) and to some degree HSP70 and HSP90. This host-directed activity interferes with the unfolded protein response and prevents the viruses from generating properly folded proteins and efficient viral assembly. The down regulation of GRP78 furthermore results in the up-regulation of PERK which induces the autophagy which facilitates the clearing of intracellular viruses and/or phagocytized unfolded proteins. An overload of the ER stress mechanism can trigger apoptosis.

The activity of AR-12 alone has been demonstrated in vitro with a variety of viruses. AR-12 exhibited an EC₅₀ of 500 nM against Ebola, Lassa, Marburg and Nipah; and demonstrated activity against amantadine resistant influenza A strain. In combination with PDE5 inhibitors AR-12 has shown in vitro activity against Chikungunya, Measles, Mumps, Coxsacki, Adenovirus, Rubella, RSV, CMV, Hepatitis A and Hepatitis B.

This novel antiviral MOA offers a new approach to treat viral infections either alone or in combination with established drugs. It also has the potential to be used in the prophylactic setting for health care workers at risk during viral outbreaks.

219 **The Lipid Kinase Sphingosine Kinase 2 is an Essential Host Factor Recruited by Chikungunya Virus During Infection**

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The Sphingosine Kinases (SKs), SK1 and SK2 are the key enzymes involved in sphingosine-1 phosphate (S1-P) production and have important roles in a wide array of biological processes. Recently, SK1 has been implicated to play role during viral infections, indicating that these kinases are likely essential host factors for viruses. By and large our understanding of SK function stems from our knowledge of SK1, in contrast relatively little is known about SK2. In the current study we identify SK2 as an essential host factor for Chikungunya virus (CHIKV) infection. Targeting of SK2 through the use of siRNAs or small molecule inhibitors significantly inhibited viral infection. We also observe that upon infection SK2 is relocalized to the viral replication complex, and the viral protein nsP3 is required for this relocalization. These data demonstrate the SK2 is a novel CHIKV host factor required for viral replication

220 **Antiviral Activity of MEK/ERK Inhibitor AZD6244 Against Dengue Virus In AG129 Mouse**

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Here we investigated the potential antiviral activity of AZD 6244 an MEK/ERK inhibitor, against D2S20, a Dengue Hemorrhagic Fever (DHF) strain. Treatment of BHK-21 cells with AZD6244 impairs replication of both DENV-2/3 and affects DENV morphogenesis as well. Analysis carried out *in vivo* with AG129 mouse, showed that pre-treatment with AZD6244 conferred a substantial and robust protection against lethal infection with D2S20 (100% survival). Together, our data support the potential use of AZD6244 as an anti-DENV therapeutic both *in vitro* and *in vivo*.

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221 **Cyclin D3 regulates CDK6 control of the viral restriction factor SAMHD1**

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Cyclins control the activation of cyclin-dependent kinases (CDK), which in turn, control cell proliferation. We have shown that the virus restriction factor SAMHD1 is controlled by CDK6, a CDK controlling early G0 to G1 transition of the cell cycle. However, the cyclin controlling the process of SAMHD1 function has not been clearly identified in primary cells. To elucidate this, human monocytes were isolated and transfected with small interfering RNA (siRNA) against eight distinct cyclins controlling the cell cycle. Transfected monocytes were differentiated to macrophages (MDM) using M-CSF and infected with a VSV-pseudotyped NL4-3 GFP-expressing virus or full-replicative R5 HIV-1 strain Bal. Downregulation of the CDK6-associated cyclin D3 showed the strongest inhibitory effect ($p=0.0002$) of HIV-1 replication and total viral DNA formation in acutely infected MDM, whereas cyclins D1 and E2 (associated to CDK6 and CDK2, respectively) had a lower inhibitory effect. Cyclin A2, B1, B2 and D2 did not block HIV-1 replication. Alternative siRNA sequences targeting cyclin D3 confirmed the effect of cyclin D3 in HIV-1 infection and total viral DNA formation.

Cyclin D3 downregulation led to a significant ($p < 0.01$) reduction of SAMHD1 phosphorylation (at residue T592), CDK2 activation as measured by phosphorylation of T160 and decreased intracellular dNTP levels. The effect of cyclin D3 RNA interference was lost after degradation of SAMHD1 by HIV-2 Vpx, demonstrating the specificity of the mechanism. Our results show that knockdown of cyclin D3 has a major impact in SAMHD1 phosphorylation, dNTP levels and HIV-1 reverse transcription and replication. Cyclin D3 is the catalytic partner of CDK6. Thus, our results indicate a fundamental role of the CDK6-cyclin D3 complex in SAMHD1-mediated virus restriction during G0 to G1 transition.

222 Biosynthesis of Methylene-cyclopropane Nucleoside Analog Triphosphates in HCMV-Infected Cells

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Human cytomegalovirus (HCMV) has detrimental effects on patients with compromised or immature immune systems. Currently approved pharmacotherapies for systemic HCMV infections carry with them several problems including the development of viral strains with decreased drug susceptibility and high incidences of adverse effects. The methylene-cyclopropane nucleoside analogs (MCPNA) demonstrate greater efficacy against HCMV when compared to ganciclovir (GCV; the current standard of therapy) without any observed increase in cytotoxicity. The mechanism of action for each of these compounds includes selective, rate-limiting activation by the viral kinase pUL97, further phosphorylation into an active triphosphate (-TP), and subsequent inhibition of the viral DNA polymerase and/or incorporation into replicating viral DNA with premature chain termination. The purpose of this study was to characterize the biosynthesis of three generations of MCPNAs into active compounds in HCMV-infected cells. Incubation of HCMV-infected cells with 10.5 mM synguanol (syn; 1st generation), 2.5 mM cyclopropavir (CPV; 2nd generation), or 4.0 mM MBX-2168 (3rd generation) (all concentrations 5xEC₅₀) resulted in a time-dependent increase in triphosphate accumulation reaching maximums of 45.5 ± 2.5 , 121.1 ± 10.7 , and 48.1 ± 5.5 pmol/10⁶ cells at 120 hours, respectively. HCMV-infected cells incubated with 25 mM GCV (5xEC₅₀) also demonstrated a time-dependent increase in triphosphate accumulation (42.7 ± 3.7 pmol/10⁶ cells at 120 hours). Thus, the accumulation of the MCPNA-TP was equal to or greater than that of GCV-TP under equivalently effective concentrations. It appears therefore that the active triphosphate metabolites of synguanol and MBX-2168 are of equal potency when compared to GCV. In contrast, CPV-TP appears to be less potent (~2x) than GCV-TP. We conclude that the capacity of the MCPNA to elicit a greater anti-viral effect when compared to GCV can be attributed to their increased affinity for and subsequent rate-limiting activation by pUL97 and not increased affinity of active compound for target.

223 Investigating the Antiviral Effects of Cannabinoids in the 2nd Decade of HAART

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BACKGROUND: Recent shifts in public policy has facilitated increased utility of cannabis in HIV positive patients. However, significant research on antiviral activities of cannabinoids remains elusive. Exogenous cannabinoids or receptor antagonists influence many cellular and systemic host responses to HIV, CB2 agonism may have clinical benefits for Kaposi's sarcoma and antiviral/anti-inflammatory effects are being explored.

METHODS: Value measurements of peer-reviewed metrics include weighted statistical valuations, inclusion of review and post-publication measures of investigative methods.

RESULTS: Researchers demonstrated the inhibition of gp-120 mediated insults in brain microvascular endothelial cells by cannabinoids. Separate research has shown the CB2 agonist, WIN55-212, suppressed HIV-1 replication in microglial cell cultures. Results from a study on the antiviral effects of cannabinoids found that cannabinoids inhibit the migration of microglial-like cells to HIV Tat. Research has suggested that medicinal use of cannabis was associated with improved adherence to ART. Additionally, researchers have identified cannabinoid receptor-mediated attenuation of CXCR4-tropic HIV infection in primary CD4+ T cells, therefore inhibiting productive infection of cell-free or cell-associated viral acquisition of HIV in resting cells. Data has also been presented that selective synthetic CB2 agonists have been shown to limit HIV infection in macrophages by acting as immunomodulators on HIV infected macrophages. Researchers have observed that CB1 was increased in HIV encephalitis brains and those with comorbidities, while CB2 was significantly increased in the white matter of HIV encephalitis brains.

CONCLUSIONS: Further research on the clinical utility and consequences of cannabinoids is necessary to identify new opportunities for cannabinoid-based drug development for HIV patients to target insults to endothelial and microglial cells. Further research on cannabinoids is anticipated in addressing HIV-associated neurocognitive disorder, suppressing inflammatory cytokines and latent pools of resting viremia in macrophages and other cell lines that are currently representing global challenges to HIV eradication.

224 **Design, Synthesis anti-HIV activity and cytotoxicity of Novel indenoquinoxaline derivatives**

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BACKGROUND: Quinoxaline is a versatile lead molecule for designing potential bioactive agents and its derivatives were reported to possess significant antiviral and anticancer activities. Present work is to investigate the anti-HIV activity and cytotoxicity of indenoquinoxaline derivatives and also studied for cytotoxicity against breast cancer cell (MCF cells) to understand the anti-HIV activity and anticancer potential of newly synthesized compounds.

METHODS: Ninhydrin react with o-phenylenediamine to yield indenoquinoxaline lead molecule. Indenoquinoxaline condensed with primary amine to gives new derivatives. Newly synthesized compounds were screened for anti-HIV activity against HIV -1 and -2 replication in MT-4 cells and cytotoxicity also investigated in mock-infected MT-4 cells. Quinoxaline derivatives tested for anticancer activity against breast cancer cell (MCF cells)

RESULTS: Newly synthesized compound IDME-2ABT inhibits replication of HIV-2 in MT-4 cells (IC₅₀: 2.55±0.23 µg/ml). All the tested compounds exhibits cytotoxicity in Adult C type T leukemia cells (MT-4 cells). All the compounds exhibits significant cytotoxicity (IC₅₀: 142-187 µg/ml) against Breast cancer cells. Indenoquinoxaline derivatives are the novel classes for lead molecules for designing potential anti-HIV and anti-cancer agents.

Table 1. anti-HIV activity, cytotoxicity of Novel indenoquinoxaline derivatives

Compounds	Virus	IC ₅₀ ^a µg/ml	CC ₅₀ ^b µg/ml	MAX PROTECTION	CTC ₅₀ ^c (µg/ml) MTT assay against MCF-7 cells
2A4PT	IIIIB	>70.30	70.30±3.25	3	----
	ROD	>70.30	70.30±3.25	3	
2ABT	IIIIB	> 11.50	11.50±0.71	1	----
	ROD	> 11.50	11.50±0.71	1	
INE	IIIIB	>13.25	13.25±0.78	2	----
	ROD	>13.25	13.25±0.78	2	
IDME-2A4PT	IIIIB	>15.60	15.50±1.77	2	145.15±4.55
	ROD	>15.60	15.50±1.77	2	
IDME-2ABT	IIIIB	>11.16	11.16±2.90	12	----
	ROD	2.55±0.23	11.16±2.90	85	
IDME-5A	IIIIB	>12.45	12.45±1.77	2	142.14±3.32
	ROD	>12.45	12.45±1.77	2	
IDMC-SAC	IIIIB	>14.83	14.83±1.53	3	149.20±6.57
	ROD	>14.83	14.83±1.53	0	
IDME-SDM	IIIIB	>34.53	13.53±12.99	3	167.90±3.30
	ROD	>34.53	13.53±12.99	3	
IDME-SMZ	IIIIB	>21.38	21.38±7.39	2	163.14±3.41
	ROD	>21.38	21.38±7.39	2	
IDMF-5G	IIIIB	>13.55	13.55±2.47	2	187.72±4.52
	ROD	>13.55	13.55±2.47	2	
IDME-AA	IIIIB	>17.35	17.35±6.01	2	146.33±4.10
	ROD	>17.35	17.35±6.01	2	
IDME-OPD	IIIIB	>124	124±0.50	10	180.39±5.51
	ROD	>124	124±0.50	10	
AZT	IIIIB	0.0015±0.0002	>25	96	----
	ROD	0.0016±0.0002	>25	72	

- a - Effective concentration of compound, achieving 50% protection of MT-4 cells against cytopathic effect of HIV.
- b - Cytotoxic concentration of compounds, required to reduce the viability of mock infected MT-4 cells by 50%.
- c - 50% cytotoxic concentrations (CTC₅₀)

225 Investigation of anti-HIV activity and HIV Integrase inhibitory activity of Polyherbal extracts

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BACKGROUND:The development of antiviral drugs has provided crucial new means to mitigate or relieve the debilitating effects of many viral pathogens. A rich source for the discovery of new HIV infection inhibitors has been and continues to be, the ‘mining’ of the large diversity of compounds from natural products. HIV integrase (IN) plays important roles at several steps, including reverse transcription, viral DNA nuclear import, targeting viral DNA to host chromatin and integration. Identification of novel inhibitors of HIV Integrase have emerged as promising new class of antiviral agents for the treatment of AIDS. Present work is to investigation of anti-HIV activity, cytotoxicity and HIV integrase inhibitory activity of various extracts of polyherbal.

METHOD: Polyherbal extracts were tested for anti-HIV activity against HIV-1 and -2 in MT-4 cells and cytotoxicity also tested against uninfected MT-4 cells. BH extracts were investigated for inhibition of HIV integrase enzymatic activity to understand the mechanism of antiviral action. All the extracts were investigated for both 3’ processing and strand transfer process of HIV-1 integrase enzymatic activity. **Results:** All the extracts exhibited inhibitory activity against HIV-1 integrase enzyme (3’P IC₅₀: 8.8-63 µg/ml and ST IC₅₀: 4.9-65 µg/ml). The ethanolic extract (BH-H-ET) displayed significant inhibitory activity against both step of HIV In enzymatic activity (3’P IC₅₀: 8.8µg/ml and ST IC₅₀: 7.5 µg/ml). The ethanolic extract (BH-H-ET) also inhibits the HIV 1 replication at the concentration of 59.30 mg/ml and Cytotoxicity was found to be more than >125 mg/ml.

CONCLUSION: All the extracts inhibit the HIV integrase enzymatic activity and ethanolic extract inhibit of HIV Virus and Integrase enzyme.

Anti-HIV and HIV integrase inhibitory activity of Herbal Formulation (BH)

Extracts	HIV Integrase activity		Anti-HIV activity	
	IC ₅₀ 3’P ^a , (µg/ml)	IC ₅₀ ST ^b , (µg/ml)	IC ₅₀ ^c (µg/ml)	CC ₅₀ ^d (µg/ml)
BH-H-ET	8.8 ± 1.5	7.3 ± 1.9	59.30±12.73	>125
BH-II-CH-S1	63 ± 15	75 ± 30	>147	147±6.56
BH-H-CH-S2	58 ± 8	39 ± 18	>145	145±4.24
BH-II-Me	42 ± 7	47 ± 13	>121	121±9.47
BH-H-P.E	>100	65 ± 23	>20.71	20.71±10.80
PH-II-PEX Ext	9.5 ± 3.2	4.9 ± 1.2	>87.08	87.08 ± 19.81

The results are IC₅₀ ± S.D, n = 3 for HIV-1 IN inhibitory activity

^aConcentration required to inhibits 3’ processing reaction (3’P), ^bConcentration required to inhibits 3’ processing reaction (ST), ^cEffective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV, ^d50% Cytotoxic concentration of compound, required to reduce the viability of mock infected MT-4 cells by 50%.

226 Design and Synthesis of Novel Isatine-3-thiosemicarbazone derivatives as novel 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 and investigation of isatin-3-thiosemicarbazone derivatives as potential inhibitors of HIV replication and HIV integrase, HIV Integrase/LEDGF interaction.

METHOD: Novel isatin-3-thiosemicarbazone derivatives were synthesized and tested for anti-HIV activity against HIV-1 and -2 in MT-4 cells. Synthesized compounds were also investigated for inhibition of HIV integrase enzymatic activity and HIV Integrase/LEDGF interaction to understand the mechanism of antiviral action.

RESULTS: Isatin-3-Semicarbazone derivative (PS 5) inhibits HIV Integrase/Lense Epithelium Derived Growth factor (LEDGF) interaction with inhibitory concentration of $42 \pm 1 \mu\text{M}$.

CONCLUSION: Isatin-3-Semicarbazone derivatives are the novel class of inhibitors of HIV IN/LEDGF interaction (protein-protein) and this lead molecule is suitable for further molecular modifications.

HIV Integrase and LEDGF inhibitory activity of Isatine-3-semicarbazone derivatives

Compounds	3' Proc. ^a (μM)	Integration ^b (μM)	LEDGF/p75-IN ^c (μM)
PS 2	>100	>100	>100
PS 3	>100	>100	>100
PS 4	>100	>100	>100
PS 5	>100	>100	>100
PS 6	>100	>100	42 ± 1
PS 7	>100	>100	>100
PS 11	>100	>100	>100

^aConcentration required to inhibits 3' processing reaction, ^bConcentration required to inhibits 3' processing reaction, ^cConcentration required to inhibits HIV IN/LEDGF interaction.

227 A771726, the active metabolite of leflunomide, as inhibitor of Junin virus replication.

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The active metabolite of the immunomodulatory drug leflunomide A771726, also known as teriflunomide, inhibits mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), which catalyzes the dihydroorotic acid conversion to orotic acid, leading to pyrimidine depletion. This drug is usually used for the treatment of multiple sclerosis and has demonstrated antiviral activity against DNA and RNA viruses (HSV-1, CMV, BKV, HIV-1, and RSV).

Arenaviruses are enveloped viruses containing a bipartite, single-stranded RNA genome, with ambisense coding strategy. Five arenaviruses are known to cause severe hemorrhagic fevers in humans, including Junin virus (JUNV) agent of Argentine hemorrhagic fever (AHF), but at present no reliable drug therapy is available.

In the present study, the antiviral effects of A771726 were assayed against JUNV determining the effective concentration 50% (EC50) value in $19.30 \pm 0.07 \mu\text{M}$ and cytotoxic concentration 50% (CC50) of $223.13 \pm 1.87 \mu\text{M}$. The effectiveness of A771726 to inhibit JUNV multiplication was not importantly affected by the initial virus inoculum, with similar dose response curves in virus yield inhibition assays performed in Vero cells in the range of 0.01-20 plaque forming units (PFU)/cell. Mechanistic studies demonstrated that addition of A771726 to infected cells between 1-4 h after virus adsorption caused a strong inhibition of virus production whereas drug treatment at later times resulted in a time-dependent decrease of the antiviral effect. Furthermore, A771726 failed to inactivate virus before cell pre-treatment.

Viral infectivity of JUNV can be restored in the presence of excess orotic acid, which is the product of DHODH in *de novo* pyrimidine biosynthesis pathway.

These results allow considering this cellular enzyme as a promising target for hemorrhagic fever arenavirus inhibition.

228 **Antivirals Design against HIV, Influenza and Ebola on the Way to Nano-Intervention in the Fusion type I Machinery**

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The HIV, Flu and Ebola viruses enter into cells, using the type I fusion machinery. It involves the env proteins (gp41, HA and GP2, resp.) that are similar by architecture of the hairpin six-helix bundle formation. This could be a relevant target for viral entry inhibitors design, independently on differences of viral families. In parallel with known approaches to interference with fusion targets via rival CHR/NHR-mimetic peptides (MP) or by antibodies (Ab), we develop synthetic oligomer-chain blockers (OCB) of the pre-hairpin fusion intermediates. The MP/Ab amino acid chains use a majority of energy for an intramolecular self-organization, keeping only minority of molecular potency for a direct binding the target. On the contrary, the synthetic OCB constructs can be optimized as much more adaptable and efficient target-binding ligands. Basing on such chains equipped by rational combinations of charge-responsible, H-bonds-capable and anchor-active sensors to the targets, we obtain the OCB generations that appear as strong HIV-1/2 entry inhibitors (SI -> 10000), possessing also an anti-influenza A/B activity in vitro (SI -> 30000). A molecular docking-co-dynamics modeling reveals that NHR3-binding energy of the OCB can be >10 folds more than aggregation energy of viral CHR with NHR3 calculated per unit of the OCB/CHR molecular mass. I.e., the stronger binding effects became achievable via the OCB chains much shorter than amino acid chains. Moreover, an ability of OCB to be programmed for not only NHR-parallel but the NHR3 – belting arrest of the pre-hairpin targets provide the essentially more efficient mode-of-inhibition against fusion (Fig.). The new pilot substances are prepared for anti-HIV/Flu/Ebola viruses evaluations

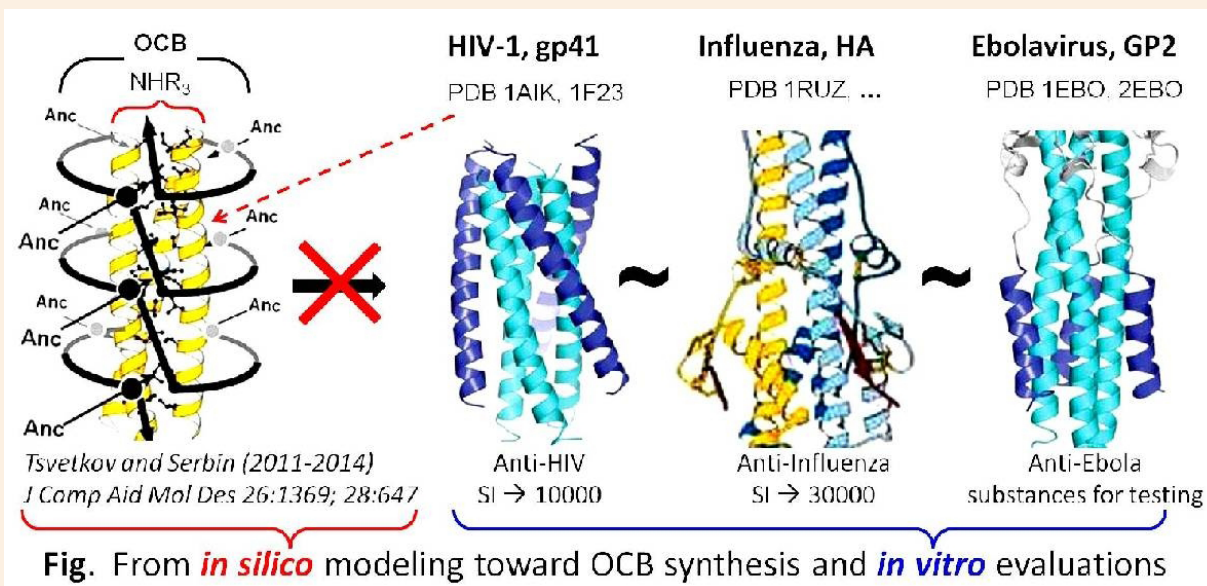


Fig. From *in silico* modeling toward OCB synthesis and *in vitro* evaluations

229 Comparison of two chemiluminescent immunoassay (CMIA I ECLIA) for determination of HbsAg, AntiHbs and AntiHbc in human serum

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INTRODUCTION: We compared the results of the COBAS e 601 (Roche) assay with Autoanalyzer Architect and SR 2000 (Abbott) assay for HBV surface antigen (HBsAg), anti-HBV surface antigen (anti-HBs) and anti-HBV core antigen (anti-HBc).

Materials and Methods: The study included 140 patients of whom 90 men and 50 women who were hospitalized at the University Clinical Centre in Sarajevo or the ambulant treated. The COBAS e 601 (Roche) uses an electrochemiluminescence immunoassay (ECLIA); serum anti-HBs is determined quantitatively while serum HbsAg and anti-HBc are determined qualitatively. The Architect i2000 analyzer (Abbott) uses a chemiluminescent microparticle immunoassay (CMIA); serum HBsAg and anti-HBs are determined quantitatively, while serum anti-HBc are determined qualitatively. Results were analyzed in SPSS 12 and Excel 2010 with a statistical significance of $p < 0.05$.

RESULTS: The concordance rates among the analyzers were 91.6%, 94.6%, and 52.2% for HBsAg, anti-HBs, and anti-HBc, respectively. The accuracy CMIA in the series is for HBsAg ranged from 3.8 to 13 % CV, and for anti-HBs is reached 2.0-6.2 CV %, and from day to day for HBsAg amounted CV 4.2 to 9.6 % for anti-HBs ranged 7.2-13 % CV. The accuracy CMIA in the series is for anti-HBc ranged from 2.6 to 6.5 % CV, and from day to day for CV 2.87-7.57 %. The accuracy ECLIA in the series is for HBsAg ranged from 1.8 to 11 % CV, and for anti-HBs is reached 1.2-4.0 CV %, and from day to day for HBsAg amounted CV 2.2 to 7.3 % for anti-HBs ranged 4.1-13.9 % CV. The accuracy ECLIA in the series is for anti-HBc ranged from 1.5 to 2.8 % CV, and from day to day for CV 1.8-8.1 %. The results showed regression line between immunoassay in patients with suspected presence of hepatitis disease HBsAg of $y = 0.604x - 110.73$; $R = 0.96$, anti-HBs $y = 0.319x + 18.47$ $R = 0.56$ and anti-HBc $y = 5,167x + 9.28$ $R = 0.82$.

CONCLUSION: The concordance rate was highest for HBsAg, followed by anti-HBs, and anti-HBc. This study shows substantial differences between the assay results of these methods, which should be taken into account in determinations of serum HBV markers.

230 Biological Response Modifiers As Enhancers of Oseltamivir Activity against Influenza Virus Type A/ H3N2 in Vivo

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Influenza continues to cause a number of morbidity cases, not rarely complicated with even a lethal outcome. Multi-target inhibition of pathology could be a reasonable approach to combat the severity of the disease. We studied the combined activity of low dosage of oseltamivir – specific anti-influenza inhibitor with proved efficacy and some potent biological response modifiers enhancing the host responses against the toxic effects of viral replication in the organism.

White male mice 18-20 g were inoculated intranasally with influenza A/Panama/2007/99 (H3N2) virus. Oseltamivir phosphate (OS) was administered *per os* in five-day-treatment course beginning 4-hours before virus inoculation with 10MLD₅₀. The antioxidant ellagic acid (EA) was applied orally once daily for five days starting 2 hours prior viral inoculation. Isoprinosine (ISO) as an immune modulator was given 10 days in two intakes with an onset the day of infection. Polyphenol complex (PC) extracted from the medicinal plant *Geranium sanguineum* was inhaled once 3 hours pre-infection. Mortality rates, protection index (PI), mean survival time (MST) and body weights were determined through 14 days post infection. Viral titers, lung index and pathology score were evaluated, too.

We observed beneficial effects of double combinations of OS 1.25, 2.5 and 5 mg/kg with PC (2.5 and 5 mg/kg), EA (500 mg/kg) and ISO (500 mg/kg) by reduction of mortality rates comparing to placebo and individually treated groups with PI up to 90% for the selected combinations. MST was prolonged up to 13.7 days as well as body weight loss was reduced. Triple combination of OS, EA and ISO also showed an increased efficacy. No significant effects were recorded for monotherapy groups. Comparison of lung parameters between treated groups also revealed the pronounced combined protection with a decrease of more than 1.66 lg CCID₅₀ titers and 1.5 lung score of selected groups.

These data suggest that antioxidants and immune modulators could enhance the protective effect of low-dose oseltamivir treatment *in vivo* against experimental infection with influenza virus A (H3N2) in mice.

231 Efficacy of Combinations of Oseltamivir and Naproxen for the Treatment of Influenza A (H1N1) Virus Infections in BALB/c Mice

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Naproxen, a known inhibitor of cyclooxygenase type 2 and an FDA-approved pain medication, was previously reported as an inhibitor of influenza virus by interaction with the viral nucleoprotein. It has antiviral properties in MDCK cells and in mice infected with influenza A viruses (Lejal et al., *Antimicrob. Agents Chemother.* 2013; 57:2231-2242). We attempted to confirm and extend these observations by evaluating naproxen alone and in combination with oseltamivir against influenza A/PR/8/34 (H1N1) infections in 6-week old female BALB/c mice infected sub-lethally with virus. Oseltamivir (administered p.o.) and naproxen (injected i.p.) treatments were given twice a day for 5 and 7 days, respectively, starting 4 h after virus exposure. Significant improvement of body weight was observed in animals treated with combinations of naproxen (100, 200 and 400 mg/kg/day) and oseltamivir (3 and 10 mg/kg/day) relative to placebos. Certain combinations of oseltamivir plus naproxen were superior to oseltamivir alone. At the doses tested, neither oseltamivir nor naproxen treatments alone was able to significantly improve body weight, although there were trends toward improvement in infected mice receiving naproxen (400 mg/kg/day) or oseltamivir (10 mg/kg/day). Lung function measurements were determined by whole body plethysmography. The best effects to improve breathing toward normalcy (using measures of enhanced pause, time of inspiration, time of expiration, relaxation time, peak inspiratory flow, peak expiratory flow, frequency of respiration, and 50% expiratory flow) were achieved by the various combinations of oseltamivir plus naproxen. Statistically significant differences were evident when comparing drug combinations to placebo, but were not generally seen when the combinations were compared to oseltamivir alone. The data indicate that naproxen will augment oseltamivir treatment of mice, although in these studies its potency as a single agent was less than that reported by other investigators. Supported by Contract No. HHSN272201000039/HHSN27200009/A56 from the Respiratory Diseases Branch, DMID, NIAID, NIH.

232 Verdinexor (KPT-335), a Selective Inhibitor of Nuclear Export (SINE) Compound, is a Broad Spectrum Inhibitor of Viral Replication

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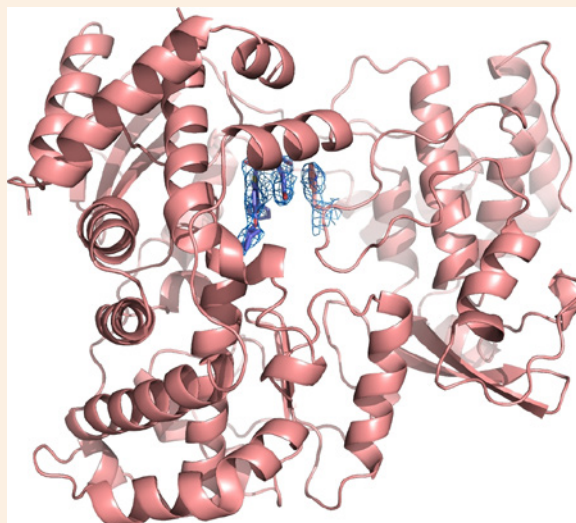
The emergence of novel and drug resistant virus strains is a global concern for public health and the development of potential bioweapons. Differences in viral life cycle, infectivity, target proteins acquired resistance make the development of broad-spectrum antiviral drugs and vaccines particularly challenging. Inhibition of host cell targets necessary for viral replication is an alternative approach that may improve efficacy and limit resistance mechanisms. Some viruses depend upon Exportin 1 (XPO1) for nuclear export of viral proteins and therefore XPO1 is a potential host cell target for novel anti-viral therapeutics. We have developed potent small molecule inhibitors of XPO1 termed SINE compounds with drugs from this class demonstrating efficacy, safety and tolerability in human and canine cancer patients. Furthermore, inhibition of XPO1 with the SINE compound verdinexor (KPT-335) has demonstrated potent antiviral activity in a number of models of influenza infection and pathology. Here we expand these findings to demonstrate SINE compound effects across a variety of plus and minus single strand RNA and double strand DNA viruses. Verdinexor was evaluated in a panel of over 40 different viruses for effects on viral replication, host cell toxicity and viral protein subcellular localization. Verdinexor demonstrated nanomolar inhibition of replication across a wide variety of viruses in the panel including HCV, HIV, HPV, Epstein-Barr and Adenovirus strains. By comparing the EC₅₀ values for antiviral activity and cytotoxicity, verdinexor showed selectivity indices for viral inhibition ranging from 3- to 3000-fold, with the greatest selectivity in influenza, HCV and HIV. Furthermore, treatment of cells with verdinexor and other SINE compounds resulted in nuclear retention of key viral proteins necessary for proper replication and assembly of infectious particles. These results demonstrate that inhibition of XPO1 with SINE compounds offers a promising new approach to targeting broad-spectrum inhibition of viral replication.

233 Targeting the flavivirus polymerase: a new class of non-nucleoside inhibitors mimicking the stacking interaction of two RNA bases

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Over the past 3 decades, many RNA virus threats were identified or “re-discovered”, including a variety of flaviviruses, like Japanese encephalitis virus, Yellow Fever virus (YFV), West-Nile virus (WNV), and Dengue virus (DENV). Infections by these neurotropic viruses may result in life-threatening encephalitis, with high risk of debilitating neurologic sequels.

Target-based design of flaviviral enzymes’ inhibitors may be a promising strategy towards antivirals development. In this context, we selected a wide region around the DENV RNA dependent RNA polymerase (RdRp) active site for *in silico* docking search of possible protein inhibitors. In this way, analyzing a proprietary library of small molecules developed by the University of Perugia, we identified the heterotricyclic compound UP4. Such compound was able to inhibit both RdRp activity and viral replication in infected cells with IC50 values in the micromolar range. The DV3 RdRp/UP4 crystal structure shows that the inhibitor interacts with the protein priming loop. Structural, biochemical and computational data suggest a non-competitive inhibition mechanism likely related to the peculiar structure of the inhibitor that mimics the stacking interaction of two RNA bases. We produced a first series of variants of the initial hit in order to start a complete SAR characterization. Some of the derivatives displays enhanced activity and reduced toxicity in cell-based assays with WNV, YFV and all the 4 serotypes of DENV.



234 Synthetic Toll-Like Receptor Ligands as Adjuvants in a Recombinant Influenza H1 Hemagglutinin Vaccine and Efficacy Against Homologous Virus Challenge in Mice

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The ability of synthetic ligands for Toll-like receptor 4 (TLR4) and TLR7 to function as adjuvants in an influenza vaccine was evaluated. 1Z105 is a substituted pyrimido [5,4-b] indole specific for the TLR4 complex, and 1V270 is a phospholipid-conjugated TLR7 agonist. The vaccine consisted of a recombinant H1 hemagglutinin (HA) combined with each ligand alone or in combination at three concentrations. Vaccine efficacy was evaluated against challenge with influenza A/CA/04/2009 (H1N1pdm) virus in mice. All adjuvant-containing vaccines provided 70-100% survival compared to the HA only vaccine given at a sub-optimal dose. Humoral immunity was determined by hemagglutination inhibition (HAI) and virus neutralization (VN) on serum collected on day 21 post-vaccination. All vaccines containing 10 nmol 1V270 provided significant HAI titers ($\geq 1:40$) compared to placebo controls, although only the vaccines containing the 10 nmol 1V270 in combination with 1Z105 provided significant VN titers ($\geq 1:128$). A dose effect was apparent, as vaccines containing 1 nmol 1V270 plus 1Z105 provided HAI titers $\geq 1:40$, but only the vaccine containing 1 nmol 1V270 plus the highest dose (200 nmol) of 1Z105 provided mean VN titers $\geq 1:128$. Differences observed in the HAI and VN results may indicate differences in the antibody binding characteristics inherent in these two functional antibody assays. Influenza virus-specific immunoglobulin levels (IgG1 and IgG2a subtypes) in serum following vaccination were evaluated by isotype-specific ELISA. Virus-specific IgG1 antibody was not detected. All vaccines containing 10 nmol 1V270 provided a significant increase in IgG2a levels compared to the HA only vaccine. Surprisingly, vaccines containing 8 or 40 nmol 1Z105 alone showed a significant decrease in IgG2a levels compared to the HA only vaccine. In summary, these data demonstrate that synthetic TLR4 or TLR7 ligands can function as adjuvants in a recombinant HA vaccine. However, 1V270 provided the best HAI antibody and virus-specific IgG2a responses. [Supported by Contract HHSN2722010000391 from the Respiratory Diseases Branch, NIAID, NIH]

235 **Creating Screening of Inhibitors of Herpes Simplex Virus Type 1 Alkaline Nuclease for molecular model**

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In order to find an effective antiviral drug against the herpes simplex virus type 1 (HSV-1) and understand its mechanism, we have screened alkaline endonuclease (AN)-dependent inhibitors. We have constructed a plasmid, pET28-UL12, with an insertion of a DNA fragment containing the UL12 gene of HSV-1 SM44, which is 99.2 % homologous to the UL12 (Gene ID: 2703382 from the GenBank), into the prokaryotic expression vector pET28a. After transformation in *Escherichia coli*, only kalamycin-resistant clones were selected. Expression of recombinant proteins in *Escherichia coli* restored the natural activity of the protein. The anti-AN activities detected in several drugs extracted from herbs were confirmed by different degrees of inhibition on the enzymatic activities, similar to the research in Vero cells that exhibited different levels of inhibiting viral infection by these drugs. The result showed that most of these drugs can dramatically interfere with HSV-1 infection on the Vero cells, but only Baicalin can significantly inhibit the rAN and stop HSV-1 replication. Docking results showed that Baicalin had a strong interaction with critical amino acid residues of AE (identity 21.12%, similar residues 41.35%, compared with rAN by DNAMAN 6.0, data not shown) which was expressed by Epstein-Barr Virus. This DNA degradation assay is a useful tool to rapidly screen the inhibitors of HSV-1 at the molecular level, also representing a potential target for novel antiviral therapies.

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236 **A cellular protein induces and stabilizes the HIV-1 LTR G-quadruplex conformation, a key regulatory element of viral transcription and new antiviral target**

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We have previously shown that the HIV-1 LTR promoter adopts multiple dynamic G-quadruplex conformations which act as a regulatory switch to control viral transcription. Compounds that stabilize the LTR G-quadruplexes were shown to inhibit viral transcription and HIV-1 infectivity. Folding and unwinding of the G-quadruplex conformations are likely controlled by interacting cellular/viral proteins. Here, by coupled EMSA and MS analysis, we identified one cellular protein that selectively binds to specific G-quadruplex LTR sequences without recognizing the LTR double-stranded or single-stranded unfolded sequence. To note, that most but not all LTR G-quadruplex forming regions were recognized by this protein, indicating both a conformation- and sequence-dependent interaction, which points to a specific activity of the protein/G-quadruplex complex at this level. Spectroscopic and biomolecular assays confirmed the high affinity of the cellular protein for the selected LTR G-quadruplex structures. The identified protein was able to both stabilize and induce LTR G-quadruplex folding, which, in turn, inhibited viral transcription. Silencing of the cellular protein by siRNAs or by a specific aptamer inhibitor greatly increased HIV-1 transcriptional levels. This study provides new insights into the mechanism of action of the LTR G-quadruplexes, which as key regulatory HIV-1 elements, represent a new potent target for antiviral therapy. Importantly, the LTR G-quadruplexes, being present at the integrated viral genome level, will allow targeting of both actively and latently infected cells.

237 HCMV UL111A and US27 Synergize to Enhance Signaling of CXCR4

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Human cytomegalovirus (HCMV) is a widespread pathogen that causes lifelong latent infection. Successful persistence of HCMV is characterized by extensive manipulation of host cellular functions and immune responses. The UL111A gene encodes cmvIL-10, an ortholog of human interleukin-10 (hIL-10), a cytokine with potent immunosuppressive properties. Despite having only 27% sequence homology to hIL-10, cmvIL-10 retains most functions of hIL-10 and acts through the hIL-10 receptor complex (IL-10R). Recently hIL-10 was found to potentiate the signaling activity of CXCR4, a human chemokine receptor that promotes migration of immune cells toward sites of injury or infection and also plays key roles in hematopoiesis and immune homeostasis. We found that cmvIL-10 can also enhance signaling of CXCR4 in response to its ligand, CXCL12. HEK293 cells endogenously express both CXCR4 and IL-10R, and these cells exhibited significantly increased calcium mobilization and migration in response to CXCL12 in the presence of cmvIL-10. Treatment with a STAT3 inhibitor or siRNA blocked the enhanced calcium flux, indicating that STAT3 activation is required for potentiation of CXCR4 by cmvIL-10. These findings demonstrate that cmvIL-10 triggers events that augment the signaling activity of a cellular chemokine receptor, most likely through receptor crosstalk. We have previously reported that HCMV US27, an orphan chemokine receptor, is the first viral receptor known to enhance CXCR4 signaling. Here, we found that cmvIL-10 and US27 together enhanced CXCR4 calcium signaling in response to CXCL12 more than either viral protein alone. In US27-transfected and HCMV infected cells, exposure to cmvIL-10 was still able to increase calcium and migration responses to CXCL12, suggesting that the two viral proteins work synergistically to enhance CXCR4 signaling. This cooperative effect may enable HCMV to manipulate CXCR4 to alter host immune responses and modify cell trafficking patterns.

238 Dipeptidyl Peptidase 4 (DPP4) In Mink Supports Entry And Replication Of Middle Eastern Respiratory Syndrome Coronavirus

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A novel coronavirus, named the Middle East Respiratory Syndrome coronavirus (MERS-CoV), was first identified in humans in 2012. MERS infections are characterized by acute respiratory distress with fatal cases often diagnosed. Continued emergence of MERS-CoV, coupled with a lack of understanding of the natural history of MERS highlights the importance of identifying therapeutics for treatment of MERS-CoV infections in humans. Like other coronaviruses, the MERS-CoV virion utilizes a large surface Spike (S) glycoprotein for interaction with and entry into the target cell. The host cell protein dipeptidyl peptidase 4 (DPP4, aka CD26) was recently identified as a cellular receptor for MERS-CoV. Our laboratory has been working to develop rational *in vitro* and *in vivo* models of MERS-CoV infection to allow better understanding of the pathogenesis and transmission potential of virus, and also to evaluate potential therapeutic and vaccine approaches to treat or prevent MERS in humans. Here we present studies focused on characterization of the MERS-CoV receptor, DPP4 in cells from the American mink (*Neovision vision*). Using several approaches we have shown a cell line derived from mink lung epithelium to be susceptible to infection by MERS-CoV. Western blot and PCR analysis of these cells demonstrate the presence of DPP4, the receptor for MERS-CoV expressed in mink, suggesting a role for this receptor in viral entry in this species. Characterization of the expression of DPP4 in mink cells reveal multiple isoforms, that show varying patterns of expression in cells in cells transfected with each DPP4 isoform using confocal microscopy. In addition, evaluation of known DPP4 inhibitors for antiviral activity against MERS-CoV reveal potential therapeutic approaches to treatment of MERS with existing, licensed compounds. Studies underway using mink as an *in vivo* model of MERS-CoV suggest it's utility in product development for MERS-CoV

239 Activity of neuraminidase inhibitors (NAIs) on influenza virus replication in the presence of pneumococcal neuraminidase A and B (NanA and NanB)

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Secondary bacterial infections with *Streptococcus pneumoniae* represent a major cause of excess morbidity and mortality during influenza. Because of functional and structural similarities, NanA and/or NanB can promote the release of virions and be targeted by NAIs. In contrast to NanA, NanB has not thoroughly been studied yet and its impact on the lethal synergism is unknown. Therefore we (i) compared the susceptibility of viral and *in vitro*-expressed NanA and NanB against NAIs (oseltamivir, zanamivir, remazol, and artocarpin) and (ii) established *in vitro* coinubation models to study viral spread and efficacy of NAIs in the absence or presence of pneumococcal NAs.

The susceptibility of the pandemic influenza A virus A(H1N1)pdm09 and both *in vitro*-expressed NAs from pneumococcal strain serotype 2 was confirmed for oseltamivir and remazol in fluorescence and hemagglutination-based NA inhibition assays. Zanamivir only inhibited the viral enzyme while artocarpin additionally inhibited NanA, but not NanB.

Results from immunohistochemical staining of viral nucleoprotein and plaque titer determination derived from the established coinubation model demonstrated that NanA as well NanB significantly increase viral spread and yield. As suggested, zanamivir efficiently blocked A(H1N1)pdm09 replication in A549 cells. Both NanA and NanB abolished its antiviral effect. In contrast, oseltamivir and remazol exerted a dose-dependent inhibition of viral replication in the presence and absence of NanA and NanB. Viral inhibition was also observed with artocarpin in the presence of NanA, but not of NanB.

In conclusion, pneumococcal NanB and NanA significantly promote influenza virus replication. Only NAIs targeting viral and both pneumococcal NAs like oseltamivir and remazol will have the potential to prevent this during coinfection.

240 Viremia reduction measurement when antiviral treatment is started at the time of peak viremia in mice may be a more appropriate mimic for conditions in the clinical setting for dengue fever

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BACKGROUND: Dengue is a global public health threat with no approved antiviral drugs or preventative vaccines in the market. Recently, we tested celgosivir, an a-glucosidase I inhibitor, in human clinical trials in Singapore¹. Although considerable efficacy of the drug in reducing the peak viremia level and mortality was demonstrated in a lethal mouse model^{2,3}, the efficacy in humans from the clinical trial of 50 patients was less prominent, suggesting that the preclinical efficacy criteria needs to be re-evaluated. **METHODS:** AG129 mice (lacking INF-a, b and g receptor) were inoculated intravenously with DENV2 clinical strains (EDEN2 isolated from patients in Singapore⁴). Drug treatment was started at the time of infection (on day 0 post-infection) or at the time of peak viremia (on day 3 post-infection) at doses of 50mg/kg BID for celgosivir or 25mg/kg BID for NITD008 (an inhibitor of DENV RdRp). The kinetics of viremia (viral copy number in serum) was measured by realtime RT-PCR. **RESULTS:** When mice were treated from day 0, viremia levels on day 3 were 9.0-fold or 4.9-fold lower than vehicle control for celgosivir ($P=0.0235$) or NITD008 ($P=0.0017$) treatment, respectively. Surprisingly, however, when the treatment was started at the time of peak viremia, the drugs failed to show significant levels of viremia reduction from day 3 to day 6 post-infection, with 281-fold (vehicle) and 400-fold (celgosivir) reduction ($P=0.434$), and 149-fold (vehicle) and 169-fold (NITD008) reduction ($P=0.450$). [Discussion] The antiviral effect appears to be less prominent once viremia reaches the peak level. It is notable that when dengue fever patients see a doctor, viremia is usually at peak level and this should be a consideration for clinical trial parameters.

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241 Competition model between nucleozin and RNA binding to Influenza nucleoprotein

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Dynamic competition between nucleozin and RNA binding to Influenza nucleoprotein

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Our previous studies have identified nucleozin as a new potential antiviral compound which aggregates the influenza A virus (IAV) nucleoprotein (NP). The IAV NP assembles with the viral RNA and polymerase subunits forming a ribonucleoprotein complex which is important for IAV replication and transcription. It is of interest to illustrate the relationships among nucleozin, NP and RNA. Here we propose a competition model to explain the involvement of RNA in nucleozin-induced NP aggregation process and illustrate how nucleozin affect NP-RNA binding process. Recombinant NP protein and derived NP variants engineered for oligomerization deficiency (E339A) or nucleozin binding deficiency (Y289H) were expressed in E.coli and purified to homogeneity. The purified NPs mainly existed as a monomer under low salt concentrations, as illustrated in dynamic light scattering (DLS). In the presence of nucleozin, more NP or E339A NP aggregates formed with the addition of small amounts of RNA, suggesting that RNA maybe important for facilitating nucleozin-induced NP aggregation. Using DLS, we were able to show that a small amount of RNA accelerated the rate of nucleozin-induced aggregation process. Upon addition of more RNA, the NP oligomers mediated by RNA and NP aggregation induced by nucleozin co-existed. Both DLS and native PAGE showed that a large excess amount of RNA would prevent nucleozin-induced NP aggregation, suggesting that the binding domains between NP and RNA or nucleozin may be partially overlapped. This dynamic competition process between nucleozin and RNA binding to NP are shown to be dependent on the length of RNA.

242 Antiviral activity of new fluorinated nucleosides against herpesvirus

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Viruses of family Herpesviridae, can cause dangerous recurrent diseases, transplacental infections with progressed of inborn defects and potentially lethal lymphoproliferative diseases. A significant increase of herpetic diseases among adults and children determine necessity of multifaceted study of such infections and development of effective methods of prevention and treatment of various forms of pathologies caused by herpesviruses. In clinical practice for treating these diseases most frequently use nucleosides, modified in heterocyclic, phosphate or carbohydrate fragments of the molecule. Pre-screening fluorinated nucleosides, synthesized at the Institute of Organic Chemistry (Ukraine), allowed found out a number of compounds that have activity against herpes simplex virus type 1 in cell culture.

The purpose of this study was to investigate the two compounds 2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazoles *in vivo*.

White mice and the reference strain of herpes simplex virus type 1 (strain US1), obtained from the Institute of Clinical Center antiviral chemotherapy and Theoretical Medicine (Germany) were used. Virus was introduced intracerebrally for modeling herpetic meningoencephalitis. Test compounds were intraperitoneal introduced at three concentrations (50, 100, 500 µg / ml) for each group of animals containing at 10 mice. Acyclovir was used as a reference drug. Survivability of animals were controlled for 14 days. Furthermore levels of γ -interferon secreted by splenocytes, isolated from the spleens of experimental animals, and in serum of animals were investigated by using the test system «Mouse IFN ELISE Kit» «Thermo scientific» (USA). It was shown antiviral activity of test compounds. In the lowest concentrations (0.4 and 0.5 mg / kg of weight) they inhibited virus replication by 90 and 100%, respectively. Increasing of γ -INF level in serum of animals was observed when test compounds were inputed to mice infected by HSV-1, that resulted to additional antiviral protection of animals. Our studies indicated that there is antihpetic action of test compounds and there is a need to in-depth study of the mechanisms of this process.

243 Flexibility of the S2 subsite in alpha- and beta-coronavirus main proteases determines susceptibility to peptidomimetic inhibitors

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Human coronavirus NL63 (HCoV NL63) belongs to the genus *-coronavirus*; it infects children and immunocompromised patients, occasionally resulting in serious lower-respiratory tract disease^[1]. The main protease (M^{pro}) of coronaviruses is an appealing antiviral target, because it processes the viral polyproteins into various functional subunits of the replication/transcription complex^[2]. The cleavage specificity of the protease (Gln-small a.a.) is unique and not found in host-cell proteases.

We have designed the Michael acceptor, SG85^[3] and the α -ketoamide lead compound DC401903 which show broad-spectrum inhibitory activities against enterovirus 3C^{pro}s and β -coronavirus M^{pro}s, but not against the HCoV NL63 M^{pro}. We determined the crystal structure of HCoV-NL63 M^{pro} and compared it with SARS-CoV M^{pro}. Differences include the deletion of Met49 and the replacement of Gln189 by Pro in the HCoV-NL63 M^{pro}. Both of these differences are located in the hydrophobic S2 pocket. In many crystal structures of the SARS-CoV M^{pro} with our inhibitors, Gln189 is flexible and adapts to the hydrophobic P2 group of the inhibitors. In contrast, the Pro at this position in HCoV-NL63 M^{pro} is not flexible enough to undergo conformational changes.

The inhibitory activity of SG85 and DC401903 against HCoV-NL63 M^{pro} improved dramatically when we introduced the mutation Pro189Gln and inserted Met at position 49, i.e. created a SARS-CoV M^{pro}-like S2 subsite within the context of the HCoV-NL63 enzyme. In order to develop broad-spectrum α -ketoamide inhibitors against both β - and α -coronavirus M^{pro}s, we designed compounds with smaller P2 moieties such as *i*-butyl. The resulting compounds show low micromolar activity against both HCoV-NL63 and SARS-CoV M^{pro}. The data are now used to further increase the affinities of these broad-spectrum inhibitors.

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244 Piceatannol Prevents HIV Entry into the Cells

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Natural products are rich sources of bioactive compounds and have shown their practical value in the therapies of many diseases. *Cassia abbreviata* is widely used in Africa to treat many diseases including HIV infection. Bark, roots and branches from *Cassia abbreviata* were collected in Kenya. Crude extract was obtained from bark after ethanol and ethyl acetate extraction. Crude extract protected MT-4 cells against HIV-1 IIIIB (X4 tropic) (IC₅₀=21.75±1.20µg/ml; CC₅₀=272.45±110.38µg/ml; SI=12.69±5.78). It also inhibited human peripheral blood mononuclear cells (PBMCs) infection by HIV-1 ADA-M (R5 tropic) (IC₅₀=13.53±12.99µg/ml) and HIV-1 IIIIB (IC₅₀=40.77±4.04µg/ml) without any toxicity (CC₅₀>1000µg/ml). Fifty compounds were purified using different solvents according to their polarity. Four compounds, oleanolic acid, palmitic acid, taxifolin and piceatannol, showed anti-HIV activity. The most potent compound, piceatannol, inhibited U373-CD4-CXCR4 cells infection by pseudotype particles pNL4.3 *EnvLuc*-HXB2 (X4 tropic) (IC₅₀=42.13±23.52µM) and U373-CD4-CCR5 cells infection by pseudotype particles pNL4.3 *EnvLuc*-Bal (R5 tropic) (IC₅₀=47.46±6.52µM, CC₅₀>1000µM). Piceatannol inhibited viral infection when added at the time of infection as well as when pre-incubated with the cells, but not with the virus, 2 hours before infection, and neither when post-incubated 2 hours after infection. These data emphasize that piceatannol might act at an early stage of HIV infection preventing HIV entry by targeting cells. Accordingly, piceatannol showed a strong synergistic activity with the fusion inhibitor Enfuvirtide (T-20) but did not bind CXCR4 or CCR5 indicating that it may interfere with other steps of HIV-1 entry. In line with this observation, piceatannol showed an inhibitory effect on pseudotype particles pNL4.3 *EnvLuc*-VSVG infection (IC₅₀=79.23±17.20µM) suggesting that it inhibits other viruses entry by a non-specific binding to the cell membrane. In conclusion, piceatannol might represent a good candidate for further development of potent antivirals.

245 Antiviral effect in influenza patients treated with metered dose inhaler containing aprotinin, a protease inhibitor

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Influenza virus is activated by host respiratory proteases to maintain infection in respiratory epithelium and pathogenesis of disease. Inhalations of aprotinin, a natural propease inhibitor, were found to provide therapeutic effect in influenza (for review see Zhirnov et al., *Antiviral. Res.* 92(1): 27-36 2011). Antiviral efficacy of inhalations of aprotinin aerosol generated by meter dose manual inhaler (MDI) were studied in influenza patients. Clinical trials were performed during outbreak in Moscow region caused with pandemic Influenza H1N1pdm09 virus. Propellant type MDI (Aerus™, Russia) containing aprotinin as an active substance was used. Patients inhaled nasally 2 aerosol doses of aprotinin (160 Kallikrein-inhibiting Units (KIU)) each 2 hours for 5 days. In comparison group, patients were treated with ingavirin™ (a synthetic peptidoamine with unknown antiviral target), 90 mg per day for 5 days. On day 2 after treatment virus loads in nasal-pharyngeal washes were determined by real time PCR. Because amounts of host cells in nasopharyngeal washes varied from patient to patient, amounts of viral RNA were normalized to host ribosomal 18S RNA determined by real time PCR with human ribosome specific primers. About 10 fold decrease of virus load in aprotinin patients were determined in comparison to ingavirin patients. Duration of clinical symptoms, such as headache, sore throat, cough, sore thorax, rhinorrhea, weakness, fever, was 1-2 days shorter in aprotinin then in ingavirin group. About 35 patients were observed and no side effects were documented in aprotinin-treated patients. Aprotinin MDI can be recommended as a drug of choice against Influenza caused by different viruses because phenomenon of virus activation by host proteases is a major pathogenesis mechanism in all influenza viruses.

246 Pharmacological Enhancement of Anti-Viral DNA Polymerase Inhibitors via Reduction of Endogenous Nucleoside Triphosphates

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Human cytomegalovirus (HCMV) infects the majority of the world's population. Although immunocompetent individuals present as asymptomatic, HCMV may prove detrimental in patients who are immunocompromised or immunologically immature. Complications from HCMV include retinitis, pneumonitis, and permanent neurological damage. The current standards of HCMV pharmacotherapy are nucleoside analogues (ganciclovir (GCV), cidofovir (CDV)) that directly inhibit viral DNA polymerase and/or compete with endogenous deoxynucleoside triphosphates (dNTPs) for incorporation into replicating viral DNA resulting in early chain termination. Cyclopropavir (CPV), a novel methylenecyclopropane nucleoside analog currently in phase 1 clinical trials, elicits an anti-viral effect via a mechanism similar to GCV. Life-long adherence to therapy is required due to recurrence of infection upon cessation of treatment. As such, the development of drug resistant HCMV strains is problematic. In this study, we explore the use of hydroxyurea (HU), an inhibitor of ribonucleotide reductase, to enhance the potency of nucleoside analogs by decreasing the cellular concentration of endogenous dNTPs. We hypothesize that a reduction in endogenous dNTPs will result in increased nucleoside analog incorporation into viral DNA manifesting in a potentiation of anti-viral effect. Standard combination viral plaque reduction assays, with a 95% confidence interval, have revealed high synergy indexes: 35.74 (GCV and HU) and 65.14 (CDV and HU). These results indicate a high synergistic relationship between nucleoside analogues and hydroxyurea. We therefore conclude that hydroxyurea synergistically enhances the anti-viral effect of nucleoside analogs used for the treatment of systemic HCMV infections. This combination therapy could assist with the treatment of HCMV strains with decreased drug susceptibility by pharmacologically enhancing the potency of the anti-viral nucleoside analogs. In addition, incidence rates of adverse effects caused by the administration of anti-viral nucleoside analogs could be reduced since a lower dose would be required to elicit the same effect.

247 Establishing *in vitro* assays and a robust mouse model to identify inhibitors of the Zika virus.

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The flavivirus Zika virus (ZIKV) (related to dengue, yellow fever or Japanese encephalitis viruses) is widely prevalent in Africa and South East Asia². An outbreak of ZIKV in French Polynesia in 2013 resulted in 30 000 people seeking medical care³. An infection with ZIKV may cause a disease known as Zika fever characterized by headaches, maculopapular rash, fever, malaise, conjunctivitis and arthralgia⁴. Several cases of patients with serious neurological complications (e.g. encephalitis, meningo-encephalitis) following infection with ZIKV, have been reported⁴. There is neither a vaccine nor a specific therapy for the treatment of infections caused by ZIKV. We report on the establishment of *in vitro* assays that may allow identifying inhibitors of ZIKV replication. ZIKV replication *in vitro* is moderately inhibited by interferon- α , ribavirin, the nucleoside analogues 2'-C-methylcytidine, 7-deaza-2'-C-methyladenosine and the influenza drug Favipiravir [T-705] and its analogue T-1105. Next a ZIKV infection model was developed in interferon α/β and γ receptor knock-out (AG129) mice. Intraperitoneal inoculation of these mice with as few as 10 PFU of ZIKV resulted in the induction of virus-induced disease (ruffled fur, body weight loss, reduced activity, paralysis) [mean day of euthanasia (MDE) 18 days post infection]. Infection with a higher inoculum (10^2 - 10^5 FPU) resulted in faster progression of the disease (MDE of 14 dpi). Infection resulted in the induction of pro-inflammatory cytokines and chemokines (IFN- γ , IL-18, IL-6, TNF- α , IP-10) detected in the serum. Histopathological analysis revealed the accumulation of viral antigens in motor neurons of both the brain and spinal cord. The acute neutrophilic encephalitis was observed at the time of onset of virus-induced morbidity. 2'-C-methylcytidine reduced viremia with about 1 log₁₀ but did not protect against ZIKV-induced morbidity and mortality. In conclusion, we established the necessary *in vitro* assays and a robust mouse model that should allow to identify inhibitors of ZIKV infections.

Clinical Evaluation of Antiviral Therapies

Chairs: **Michael Manns, M.D.**

8:30 AM – 10:00 AM

ESTENSI – MEDICI

249 Discovery and Characterization of MK-8876, a Novel Non-Nucleoside Inhibitor of HCV NS5B Which Possesses Broad Genotypic Potency

Steven W. Ludmerer, Casey McComas, Anandan Palani, Joseph J. Salata, Ellen Hulskotte, Maureen Ho, Arthur Simen, Joan R. Butterton

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This work describes the discovery of MK-8876, a potent non-nucleoside inhibitor of HCV NS5B with broad *in vitro* activity across genotypes and known non-nucleoside inhibitor resistant variants, and will detail its preclinical and clinical profile. A concerted medicinal chemistry effort sought to capture greater binding interactions within a central pocket on the NS5B surface between the 'palm' and 'thumb' domains, chemically extending a central benzofuran core previously shown to bind within this pocket, and ultimately leading to the discovery of MK-8876. MK-8876 demonstrated potent inhibitory activity across broad panels of HCV genotypes and NNI mutations (eg. EC₅₀ = 1.2 nM (gt1a), 6.6 nM (gt2a), 1.6 nM (gt3a), 1.2 nM (gt4a), 3.4 nM (gt1a C316Y), 2.9 nM (gt1a S365A), little resistance following direct *in vitro* resistance selections, and a favorable pharmacokinetic profile in both rat and dog, with good bioavailability and AUC_{0-24h} (mM.hr) of 13.5 (rat) or 12.7 (dog) following oral dosing at 10 and 5 mpk respectively. Given this favorable profile, MK-8876 entered PhI clinical trials which included single and multiple-dose escalation studies and a 7-day PhIb proof-of-concept study in gt1a or gt3 patients. In the 7 day monotherapy study evaluating MK-8876 at 800 mg once-daily, the mean maximal viral load reductions (log₁₀ U/mL) were -3.39 (gt1a) and -1.88 (gt3). The differential response between gt1a and gt3 patients was not anticipated by the *in vitro* potency profile, nor could be explained by MK-8876 plasma pharmacokinetics, which were comparable between genotype panels. A previously unobserved S365L mutation arose during viral rebound in all three gt1a patients, in two during the dosing interval and correlating with a partial rebound in viral titer. Among the gt3 patients no mutations arose during the study nor were any mutations previously associated with NNI drug resistance noted at baseline or during the study. MK-8876 demonstrated promising pan-genotypic activity in PhI studies, and its novel chemical scaffold enables further optimization to achieve broadly active non-nucleoside inhibitors.

250 Quantitative mutant analysis of naturally occurring V-073-antiviral resistant polioviral quasispecies using the MAPREC-assay in infants receiving OPV

Kimberley S.M. Benschop¹, Joost Verhoeven¹, Gokhan Uslu¹, Edin Jusic¹, Erwin Duizer¹, Marion P.G. Koopmans², Harrie G. van der Avoort¹

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Background. Pocopavir (previously V-073) has been shown to effectively inhibit the replication of wild type poliovirus (PV), vaccine oral poliovirus (OPV), and vaccine derived poliovirus (VDPV) of the 3 serotypes. Drug resistant virus variants could be identified by drug selection; resistant markers were a phenylalanine replacement of isoleucine at amino acid residue 194 (192 in PV3) in the VP1 capsid protein (I194F) and an alanine to valine change at position 24 in the VP3 capsid protein (A24V). We identified 6 natural resistant OPV isolates containing the I194F change and one strain containing the A24V change isolated from 7 newborns participating in a poliovirus vaccine trial. Objective and methods. In order to investigate the quasispecies occurrence of resistance under natural conditions we developed a Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) for the I194F marker. The MAPREC assay quantifies the viral population with this specific marker. Consecutive sampling during a period of 2 months, yielded 16 isolates from the 7 newborns. Results. Using the MAPREC assay we were able to identify minority populations already present (< 25%) in 9 isolates before resistance was phenotypically observed. In 6 isolates the majority (>95%) of the viral population could be characterized to carry the I194F marker, and were found to be phenotypically resistant (IC₅₀ > 1 μM). In one strain resistance was also observed phenotypically with a I194F-viral population >58%. We also identified a minority population in two vaccine batches (1 and 11%). Conclusion. In this study, we report the use of the MAPREC assay to quantify the presence of resistant quasispecies viral populations against pocapavir under natural conditions. These data also show a possible threshold to exist for minority mutant populations to be phenotypically characterized by cell culture methods. Further studies are needed to determine whether such thresholds can be used to predict drug response and phenotypic resistance development before virus clearance is achieved.

Animal Models of Infection

Chairs: **Don Smee, Ph.D.**

10:30 AM – 12:00 PM

ESTENSI – MEDICI

252 Experimental Respiratory Infection of Hartley Guinea Pigs with Ebola Virus Zaire

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Ebola virus is a negative-stranded, enveloped RNA virus and a member of the Filoviridae family. It causes severe viral hemorrhagic fevers with case-fatality rates exceeding 50%. Currently, there are no licensed medical countermeasures against filoviruses. Therefore, it is important to have a small animal model for these viruses, especially for the aerosol route of exposure which could be highly infectious. In this study, we exposed two groups of 10 naïve Hartley guinea pigs to target doses of 10 PFU (low dose) and 1000 PFU (high dose) of aerosolized guinea pig adapted Ebola virus Zaire strain (EBOV). Guinea pigs started to show clinical signs of the disease including weakness, fever, anorexia, and weight loss on day 4 post-exposure (PE). While the high dose exposure group started to show increased body temperature on day 4 PE, the low dose exposure group did not have increased body temperature until day 5 PE. Blood was collected on day -7 and then on days 3, 6, and 9 PE to test complete blood count and serum chemistries. Interestingly, as white blood cells, lymphocytes, and platelets decreased, monocytes increased throughout the course of the disease. Increases in liver enzymes such as alkaline phosphatase, alanine transferase, and aspartate transferase, as well as total bilirubin were observed beginning on day 6 PE. Furthermore, blood urea nitrogen and creatinine levels were high in moribund animals. Low dose and high dose exposure groups succumbed to disease at an average of 9.0 ± 0.48 and 8.3 ± 1.0 days PE, respectively. Histopathology revealed that all of the animals developed systemic viral infections and had lesions in the liver, spleen, adrenal glands, intestines, and reproductive tract that were characteristic of those that occur in guinea pigs infected subcutaneously or intraperitoneally with EBOV. However, in contrast to other routes of viral infection, the aerosolized virus caused significant lesions in the respiratory tract – primarily in the lungs and draining lymph nodes.

253 A Novel Dengue Model in STAT2 Knockout Hamsters

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Dengue virus (DENV) is an emerging flavivirus that causes disease throughout tropical areas of the world with an estimated 390-million dengue infections per year with no available vaccines or antivirals. One of the main reasons for lack of interventions for dengue is the lack of an appropriate animal model that replicates clinical disease. The interferon receptor-deficient AG129 mouse strain is commonly used in DENV studies, but this mouse strain is highly immune compromised and generally requires intravenous challenge with high-titered virus in order to cause severe disease. We recently succeeded in the establishment of gene targeting technologies for the golden Syrian hamster, allowing for the first time, the production of genetically modified hamsters. Employing these newly developed gene-targeting tools, we have created STAT2 knockout (KO) hamsters. These STAT2 KO hamsters display increased sensitivity to dengue infection when compared with an AG129 mouse model. Lethal disease, including weight loss, vascular permeability, and other relevant disease parameters, was observed after intraperitoneal inoculation with 10⁶ CCID₅₀ of the New Guinea C strain of DENV-2. Another strain of DENV-2 also produced similar disease. Infection of AG129 mice in parallel studies generally resulted in reduced disease severity, CNS infection or no disease manifestation. We anticipate that infection of STAT2 KO hamsters with DENV represents a useful model of disease with greater immune competence and increased susceptibility over currently available small animal models.

254 Connecting the dots: Study of West Nile virus spread within the Central Nervous System of primates as a foundation for development of therapeutic interventions for flaviviral encephalitis

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West Nile virus (WNV) is a mosquito-borne neurotropic flavivirus that is emerging as a human pathogen of global scale. Currently, there are no approved vaccines or treatments for WNV infection. Spread of the WNV from the site of mosquito bite to the central nervous system (CNS) is believed to follow two possible routes: hematogenous and/or axonal transport along the peripheral nerves. However, the mode of virus spread once in the CNS and the reason for its “tropism” to particular CNS structures remain elusive. To determine patterns and possible mode of WNV spread within the CNS, we analyzed virus loads and anatomical localization of WNV-infected neurons in the entire CNS of non-human primates following bilateral intrathalamic inoculations of the virus. Intraneuronal localizations of viral particles and sites of virus replication were determined by electron microscopy. Connectogram design was used to visualize the neuroanatomical connectivity between regions that harbored WNV-infected neurons and to reconstruct the spread of WNV within the CNS. Our results show that WNV preferentially uses the structures and pathways of motor control system for replication and transneuronal spread within the CNS. Connectogram reconstruction of virus spread and ultrastructural analysis suggest that WNV can utilize both anterograde and retrograde transneuronal transport to travel within vesicles along the microtubular system and infect synaptically connected neurons. Combining examination of neuroanatomical connectivity with analyses of viral replication and topography of infected neurons, such as done in this study, might provide a model and new insights into neuropathogenesis of flavivirus infections. This, in turn, might guide the development of drugs and/or therapeutic interventions to help treat flavivirus neuroinfection.

255 In Vivo Antiviral Efficacy of VerdineXor in Two Animal Models of Influenza A Virus Infection

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Influenza virus causes seasonal epidemics of contagious respiratory illness that can lead to mild to severe illness, and at times, death. Vaccination is the principal strategy for controlling influenza virus despite variable vaccine efficacy. Influenza antiviral drugs are an important adjunct to vaccination; however, substantial drug resistance has developed to two of the four currently approved antiviral drugs. Thus, new therapeutic approaches are being sought to reduce influenza-related disease burden. Previously, we have demonstrated the antiviral efficacy of verdineXor, and inhibitor of the nuclear export protein XPO1, in suppressing influenza

replication *in vitro* and virus burden in infected mice. As viral RNAs are replicated inside the nucleus of infected cells, verdinexor acts to inhibit viral ribonucleoproteins (vRNPs) export from the nucleus, thereby blocking virus replication. Furthermore, verdinexor was shown to be efficacious against various types, subtypes, and strains of influenza virus. In the present study, *in vivo* efficacy of verdinexor was further evaluated in mouse and ferret models of influenza infection. In mouse, verdinexor was efficacious to limit virus shedding, pro-inflammatory cytokines secretion, and moderate inflammation in the bronchoalveolar space. Verdinexor-treated ferrets displayed reduced lung pathology, virus burden, and inflammatory cytokine expression in the nasal wash exudate. These findings further demonstrate antiviral efficacy of verdinexor and support its current development as a novel antiviral therapeutic for influenza.

verdinexor (KPT-335)

in vitro*

- efficacious against various influenza A & B strains
- inhibits viral nuclear export
- retention of NFκB subunit p65 in the nucleus

*previously published (Perwitasari, et al., 2014)

in vivo

	Mouse 	Ferret
<ul style="list-style-type: none"> - reduces lung and BAL virus titers - improves survival and lung pathology* - limits inflammation (cytokines and immune cells infiltration) - efficacious even at delayed dosing 	<ul style="list-style-type: none"> - reduces lung virus titer - improves lung pathology - limits inflammation (cytokines) 	

256 Antiviral efficacy of an RSV fusion inhibitor in a bovine model of RSV infection

Robert Jordan¹, Matt Shao², Richard L. Mackman¹, Michel Perron¹, Tomas Cihlar¹, Mark L. Anderson², Heather McEligot², Laurel J. Gerschwin²

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Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis and pneumonia in infants and multiple adult patient populations. Effective treatment for RSV infection is still a significant unmet medical need. While new RSV therapeutics are in development, there are few animal models that mimic the pathogenesis of human RSV, making it difficult to evaluate new disease interventions. Experimental infection of Holstein calves with bovine RSV (bRSV) causes a severe respiratory infection that is similar to human RSV infection, providing a relevant model for testing novel therapeutic agents. In this model, viral load is readily detectable in nasal secretions by qRT-PCR and cumulative symptom scoring together with histopathology evaluations of infected tissues allow for the assessment of disease severity. The bRSV model was used to evaluate the antiviral activity of a fusion inhibitor, GS1, which blocks virus entry by inhibiting the fusion of the viral envelope with the host cell membrane. The efficacy of GS1, a close structural analog of GS-5806 that is being developed to treat RSV infection in humans, was evaluated in 2 randomized, blinded, placebo controlled studies in bRSV-infected calves. Intravenous administration of GS1 at 4 mg/kg/day for 7 days starting at 24 h or 72 h post-inoculation reduced viral load, symptom scores, and respiratory rates in both studies. In Study 1, mean AUC values for viral load in nasal secretions for GS1-treated and placebo animals administered at 24 h post-inoculation were 5.9 and 18.9 log₁₀ bRSV/GAPDH equivalents×day/mL (p = 0.061), respectively. The mean AUC of symptom scores in GS1-treated and placebo animals were 366.3 and 1912.4 score×day (p = 0.018), respectively. A decrease in respiration rates among treated animals compared to placebo animals was also noted (293.0 vs. 508.6 breaths×day/min; p = 0.027). Lung pathology associated with bRSV infection was markedly reduced in GS1-treated animals compared to placebo animals. These data support the use of the bRSV model for evaluation of experimental therapeutics for treatment of RSV.

257 **Cidofovir, Ganciclovir, and Valcanciclovir Inhibit Human Adenovirus Replication and Toxicity in Permissive Immunosuppressed Syrian Hamsters**

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Saint Louis University, St. Louis, USA

Adenovirus (Ad) infections of immunocompromised patients can develop into deadly multi-organ or systemic disease. At present, there is no drug approved worldwide for treatment of Ad infections. In order to study disseminated Ad infections in immunosuppressed patients, we have developed an immunosuppressed Syrian hamster model that is permissive for replication of human Ads in Species C. Hamsters are immunosuppressed with high dose cyclophosphamide and then infected intravenously with $\sim 10^{10}$ PFU of wild-type Ad5. Ad5 replicates to high levels in the liver and other organs, and the hamsters develop symptoms similar to those seen in humans. We report that cidofovir, ganciclovir (GCV), and valganciclovir (VGCV) are effective against replication of human Ads in Species B, C, and E in cell culture. We show that clinically relevant doses of cidofovir, GCV, and VGCV are effective against Ad5 in our Syrian hamster model when used prophylactically or therapeutically (starting at day 1, 2, 3, or 4 postinfection). The drugs reduce body weight loss, greatly decrease Ad5 replication in the liver, reduce the levels of serum aminotransferases, decrease liver histopathology, and increase survival of the hamsters. We further report that Ad6 (a Species C Ad) is much more pathogenic than Ad5 in our hamster model, with an LD50 that is 10-fold lower than that of Ad5; despite this, VGCV is very effective against Ad6. We show that GCV and VGCV inhibit Ad5 DNA synthesis and late gene expression in cell culture. With herpes simplex virus and human cytomegalovirus, GCV and VGCV require initial phosphorylation by a viral-coded kinase, then the triphosphate form of the drugs is used as a substrate by the viral DNA polymerase, leading to daughter strand DNA chain termination. With Ad5, the mechanism of action for GCV and VGCV is not clear. We show that GCV is not phosphorylated in Ad5-infected cells. Preliminary data suggest that they exert their anti-Ad effect by disturbing intracellular nucleotide pools. Also, *in vitro*, GCV and VGCV appear to inhibit the polymerase activity of purified Ad5 DNA polymerase. Cidofovir, GCV, and VGCV are drug candidates for treatment of Ad infections.

258 **FILOVIR and INFLUENZAVIR: new online resources for the virologist**

Luca Zinzula¹, Cristian Romagnani², Massimiliano Orsini³

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Websites on selected groups of viruses tend to lack an interface with the social-networking and micro-blogging world. We therefore created the websites FILOVIR (www.filovir.com) and INFLUENZAVIR (www.influenzavir.com) to collect information from the most authoritative open-source databases and research tools dedicated to Ebola and Marburg viruses and to influenza viruses. In compliance with copyright restrictions and with the authorization of the original sources, external web-pages are *i-framed* into the website template to create a monothematic virtual environment, in which every resource and tool is readily accessible to the visitor. The contents are intended for scientific researchers in the broadest sense, and include bioinformatics data on the epidemiology, ecology, taxonomy, molecular biology and pathogenesis of these important viruses. Announcements of the latest scientific publications, upcoming scientific conferences and outbreak alerts are provided in real time. Accurate scientific news and media content related to filoviruses and influenza are also delivered via the Twitter pages @filovir and @influenzavir and on the YouTube channels [youtube.com/user/filovir](https://www.youtube.com/user/filovir) and [youtube.com/user/influenzavir](https://www.youtube.com/user/influenzavir). FILOVIR and INFLUENZAVIR are valuable platforms for dynamic, integrated computer-based research in virology.



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29th International Conference on Antiviral Research (ICAR)



La Jolla, CA
April 17- 21
2016


hosted by the
**INTERNATIONAL
SOCIETY FOR
ANTIVIRAL
RESEARCH
(ISAR)**

The 29th International Conference on Antiviral Research (ICAR), hosted by the International Society for Antiviral Research (ISAR), will take place at the Hilton La Jolla, La Jolla, CA. The conference will begin on Sunday, April 17, 2016, and will conclude on Thursday, April 21, 2016.

ICAR provides an interdisciplinary forum of interest to chemists, biologists, and clinicians involved in antiviral research. In 2016, scientists worldwide working in the areas of basic, applied, and clinical research will meet in a collaborative and collegial atmosphere to review recent developments in all areas of antiviral drug discovery and development.

Specific topics to be covered in the scientific program include:

- **Medicinal chemistry**
- **Virus replication**
- **Host cell-virus interactions**
- **Virus latency**
- **New target identification**
- **Biochemistry and mechanism of action**
- **Mechanisms of viral drug resistance**
- **Assay development**
- **In vitro evaluation**
- **Animal models**
- **Pharmacokinetics**
- **Toxicology**
- **Clinical trials**



The San Diego area of La Jolla is an ideal location for ICAR. It has numerous local attractions, with the coastline being one of the most popular attractions. Further, this region has a long tradition of scientific innovation, making it the ideal location for ICAR.

Visit the ISAR Web site at www.isar-icar.com to learn more about the 29th ICAR.

Abstract submissions will begin October 2015. If you have any questions, please do not hesitate to contact the ISAR/ICAR Office at 202-973-8690 or by email at ISAR@courtesyassoc.com.



ISAR INTERNATIONAL SOCIETY FOR ANTIVIRAL RESEARCH

ISAR, founded in 1987, aims to bring together the whole antiviral-research community, many disciplines (chemists, biologists, and clinicians), working in basic, applied and clinical research on antivirals, vaccines and enhancement of host defences. Members work at government agencies, pharmaceutical companies (large and small), universities etc. The society's main event is the annual International Conference on Antiviral Research (ICAR) at which the constant focus has been to inform attendees of the recent key advances in all areas of antiviral research (see next page for details of the 28th ICAR in Rome).

ISAR Member Benefits

- ▶ Discount on registration costs for members at the annual ICAR (currently \$150)
 - ▶ Reduced subscription rates to ISAR-sponsored Journals (Antiviral Research, Antiviral Therapy)
 - ▶ Quarterly ISAR Newsletters
 - ▶ Membership Directory
 - ▶ Travel Awards for qualifying ISAR members to the ICAR
 - ▶ Awards (currently \$1000 and \$500) for best submitted abstracts at the ICAR
- ...and More!

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

Specific topics to be covered in the scientific program include:

- Medicinal chemistry
- Virus replication
- Host cell-virus interactions
- Virus latency
- New target identification
- Biochemistry and mechanism of action
- Mechanisms of viral drug resistance
- Assay development
- In vitro evaluation
- Animal models
- Pharmacokinetics
- Toxicology
- Clinical trials



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Membership Rates

- 1 Year Membership \$50
- 2 Year Membership \$90
- 3 Year Membership \$120
- Students and Post-Docs \$25 Yearly

- ▶ Interdisciplinary expertise
- ▶ Collaborations
- ▶ Facilitate Networking
- ▶ Synergize Antiviral Research & Drug Discovery

Engage

Chemists • Clinicians
Biologists • Many Others

Enable

Interdisciplinary Research

Enhance

Antiviral Research & Drug discovery

Get Involved in ISAR

- Program Committee
- Conference Committee
- Finance Committee
- Career Development Committee
- Nominations Committee
- Publications Committee
- Women in Science Committee
- Poster Awards Committee
- Scientific Excellence Awards Committee
- Website Committee

Want to learn more about joining a committee?

Contact us at ISAR@courtesyassoc.com for more information.