Hosted by the International Society for Antiviral Research (ISAR)

31st International Conference on Antiviral Research (ICAR)

June 11-15, 2018

ALFÂNDEGA CONGRESS CENTRE

Porto, Portugal

Program *and* Abstracts

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- Career Development Panel

THURSDAY, JUNE 14, 2018

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- Cytomegalovirus Session
- Medicinal Chemistry Session
- Annual ISAR Business Meeting
- William Prusoff Young Investigator Award Lecture
- HIV Treatment and Cure Session
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- Shotgun Oral Presentations
- Emerging Infections and Clinical Evaluation of Antivirals Session
- DNA Viruses Session

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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 thirty-first year of existence, and has more than 300 members representing 30 countries. Membership application forms will be available at the Conference Registration desk, or from our website at **www.isar-icar.com**.

Contributors

Confirmed Sponsors as of May 28, 2018

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The EU H2020 ANTIVIRALS Consortium*

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Keynotes *and* Networking

KEYNOTE ADDRESSES

Monday, June 11, 2018

Epidemic Arboviral Diseases: Implications for Research and Public Health Infrastructure

Annelies Wilder-Smith

Umeå University, Sweden and WHO, Geneva

3:45 PM

ARCHIVE HALL I

Hepatitis C Virus: Problem Solved. And Now?

Jean-Michel Pawlotsky

University of Paris-Est

4:30 PM

ARCHIVE HALL I

NETWORKING EVENTS

Monday, June 11, 2018

Women in Science Roundtable

12:00 PM – 1:45 PM

NOBLE HALL

Opening Reception

6:45 PM – 8:00 PM

NOBLE HALL

Wednesday, June 13, 2018

New Member and First Time Attendee Networking featuring a PechaKucha Competition

12:30 PM – 1:30 PM

INFANTE HALL

Career Development Panel

1:45 PM – 2:45 PM

INFANTE HALL

Thursday, June 14, 2018

Closing Reception and Banquet

7:00 PM

FERREIRA CELLARS

(Advance purchase of banquet ticket required; transportation from select hotels provided)

Schedule *at a* Glance

MONDAY, JUNE 11, 2018

TIME	EVENT	LOCATION
11:00 AM – 6:30 PM	Registration	ARCHIVE HALL FOYER
12:00 PM – 1:45 PM	Women in Science Roundtable	NOBLE HALL
2:00 PM – 3:45 PM	Satellite Workshop of the EU H2020 Consortium 'ANTIVIRALS'	ARCHIVE HALL I
3:45 PM – 5:15 PM	Welcome and Keynote Addresses	ARCHIVE HALL I
5:15 PM – 5:45 PM	Coffee Break	RIBEIRA
5:45 PM – 6:35 PM	Recent Technological Advances Session	ARCHIVE HALL I
6:45 PM – 8:00 PM	Opening Reception *Light hors d'oeuvres served*	NOBLE HALL

TUESDAY, JUNE 12, 2018

TIME	EVENT	LOCATION
8:00 AM – 5:30 PM	Registration	ARCHIVE HALL FOYER
8:30 AM – 9:00 AM	Women in Science Award Lecture	ARCHIVE HALL I
9:00 AM – 10:00 AM	Virus Evolution Session	ARCHIVE HALL I
10:00 AM – 10:30 AM	Coffee Break	RIBEIRA
10:30 AM – 12:15 PM	Emerging Infections Session	ARCHIVE HALL I
12:15 PM – 1:30 PM	Lunch *Available for purchase*	NOBLE HALL
1:30 PM – 2:35 PM	Influenza Virus Session	ARCHIVE HALL I
2:35 PM – 3:15 PM	Coffee Break	RIBEIRA
3:15 PM – 5:00 PM	Respiratory Viruses Session	ARCHIVE HALL I
5:00 PM – 7:00 PM	Poster Session 1 *Light hors d'oeuvres served*	ARCHIVE HALL II

WEDNESDAY, JUNE 13, 2018

TIME	EVENT	LOCATION
8:00 AM – 2:30 PM	Registration	ARCHIVE HALL FOYER
8:30 AM – 9:00 AM	Antonín Holý Memorial Award Lecture	ARCHIVE HALL I
9:00 AM – 9:10 AM	*Antiviral Research:* Annual Journal Summary	ARCHIVE HALL I
9:10 AM – 10:00 AM	Viral Hepatitis Session	ARCHIVE HALL I
10:00 AM – 10:30 AM	Coffee Break	RIBEIRA
10:30 AM – 12:15 PM	Hepatitis and Retroviruses Session	ARCHIVE HALL I
12:30 PM – 1:30 PM	New Member and First Time Attendee Networking featuring a PechaKucha Competition *Light lunch served*	INFANTE HALL
1:45 PM – 2:45 PM	Career Development Panel *Snacks and beverages served*	INFANTE HALL

THURSDAY, JUNE 14, 2018

TIME	EVENT	LOCATION
8:00 AM – 5:00 PM	Registration	ARCHIVE HALL FOYER
8:15 AM – 9:00 AM	Gertrude Elion Memorial Award Lecture	ARCHIVE HALL I
9:00 AM – 10:30 AM	Cytomegalovirus Session	ARCHIVE HALL I
10:30 AM – 11:00 AM	Coffee Break	RIBEIRA
11:00 AM – 12:15 PM	Medicinal Chemistry Session	ARCHIVE HALL I
12:15 PM – 12:30 PM	Annual ISAR Business Meeting	ARCHIVE HALL I
12:30 PM – 1:30 PM	Lunch *Available for purchase*	NOBLE HALL
1:30 PM – 2:00 PM	William Prusoff Young Investigator Award Lecture	ARCHIVE HALL I
2:00 PM – 3:45 PM	HIV Treatment and Cure Session	ARCHIVE HALL I
3:45 PM – 5:45 PM	Poster Session 2 *Light hors d'oeuvres served*	ARCHIVE HALL II
7:00 PM – 10:30 PM	Closing Reception & Banquet **Advance purchase of banquet ticket required* *Transportation provided from select hotels*	FERREIRA CELLARS

FRIDAY, JUNE 15, 2018

TIME	EVENT	LOCATION
8:00 AM – 12:00 PM	Registration	ARCHIVE HALL FOYER
8:30 AM – 9:00 AM	Shotgun Oral Presentations	ARCHIVE HALL I
9:00 AM – 10:30 AM	Emerging Infections and Clinical Evaluation of Antivirals Session	ARCHIVE HALL I
10:30 AM – 11:00 AM	Coffee Break	RIBEIRA
11:00 AM – 12:30 PM	DNA Viruses and Emerging Viruses Session	ARCHIVE HALL I
12:30 PM	Conference Concludes	

2018 ISAR Award Winners

Gertrude Elion Memorial Lecture Awardee

Paul Griffiths, MD

Paul Griffiths is Professor of Virology at University College, London. He is Editor-In-Chief of Reviews in Medical Virology. His research concerns cytomegalovirus infection, where he has helped to define the natural history and pathogenesis of this infection and used this information to design randomised controlled trials of antiviral drugs and prototype vaccines.

Antonín Holý Memorial Lecture Awardee

Chris Meier, PhD

Prof. Meier, born 1962 in Berlin, Germany, received a diploma and a doctorate (PhD) in Chemistry from the University of Marburg, Germany. In his PhD thesis, he worked on the synthesis of so-called ultimate carcinogens formed by metabolic steps from aromatic amines and which are involved in the induction of the chemical carcinogenesis in the group of Prof. Gernot Boche. He joined the Organic Chemistry Division at the Pasteur-Institute in Paris, France headed by Prof. Jean Igolen and Prof. Tam Huynh-Dinh as a Post-Doc and started working on nucleoside chemistry and prodrugs. He returned to Germany joining the University of Frankfurt/Main in 1991 as an Assistant Professor under the mentorship of Prof. Joachim Engels. In 1996 he obtained the Habilitation in Organic Chemistry from the University of Frankfurt/Main, Germany. He was appointed as Associate Professor at the University of Würzburg, Germany and then in 1999 he joined University of Hamburg, Germany as a full professor.

He is the current president elect of the International Society on Nucleoside, Nucleotides and Nucleic Acids (IS3NA) and is the Scientific Director of the Centre for Structural Systems Biology (CSSB) in Hamburg. Recently he was awarded as being a Zhiquiang-Guest professor from Shanghai University, China. Before that he was an invited guest professor and visiting professor at the University of Montpellier II and Toulouse, France and Shanghai, China. His research focuses are pronucleotide development, structure-based drug design of small molecule antivirals against *Bunya viridae* and hemorrhagic fever viruses, carbohydrate chemistry, phosphorylation methods in nucleoside chemistry and the synthesis of photocaged compounds, e.g. second messengers. He has published more than 220 scientific publications and is the inventor of 10 issued patents.

William Prusoff Young Investigator Awardee

Ester Ballana, PhD

Ester Ballana is an associate researcher at IrsiCaixa-IGTP AIDS Research Institute in Badalona, Spain. She graduated in Biology from the Autonomous University of Barcelona (UAB) in 2001 and completed her PhD in Health and Life Sciences at the Centre for Genomic Regulation (CRG) in May 2007. In June 2007 she joined IrsiCaixa as a post-doctoral researcher, focusing her research on determining the contribution of host genetic factors in viral replication and disease. In 2015 she was awarded with a tenure track position from Spanish Health Ministry (Miguel Servet). Since then, she is leading a research team focused on the study of the coordinated regulation of nucleotide metabolism, viral infection and cell cycle initiation/progression, with the ultimate goal to identify markers of progression and/or therapeutic response to human diseases.

Ester's scientific and professional career has been based on the study of biological processes associated with health and disease, and she has relevant experience in several areas of biomedicine, such as genetics and genomics and virology and immunity. She has co-authored over 55 papers in international peer-reviewed journals (index h: 19, average number of citations: 22.87) and is currently supervising 3 PhD thesis. She has participated in numerous international conferences and workshops and has been involved in several Spanish- and European-funded collaborative projects. She serves as ad hoc reviewer for several scientific journals Antiviral Research, The Lancet HIV, Retrovirology, Human Immunology and Infection and Genetics and Evolution and has recently joined the editorial board of Antiviral Research. She also participates as evaluator of research projects from the Spanish National Agency for the Evaluation of Research (ANEP) and as a panel member of the expert commission for the Catalan Strategic Plan for Research and Innovation in Health (PERIS).

Women in Science Awardee

A. Desiree LaBeaud, MD, MS

Desiree LaBeaud is a physician-scientist, epidemiologist, and associate professor for the Division of Pediatric Infectious Diseases at Stanford University's School of Medicine. She received her MD from the Medical College of Wisconsin, and trained with the Rainbow Babies & Children's Hospital pediatric residency program and the pediatric infectious disease fellowship program at Case Western Reserve University, while earning her master's degree in Clinical Research and Epidemiology. Dr. LaBeaud studies the epidemiology and ecology of domestic and international arboviruses and emerging infections, with an interest in the vector, host, and environmental factors that affect transmission dynamics and spectrum of disease. Her current field sites include Kenya, Grenada, and Brazil. She currently heads a clinical research lab focused on better understanding the risk factors and long-term health consequences of arboviral infections and the most effective means of prevention. She was elected as Chair of the American Committee on Arthropod-Borne Viruses in 2017 and was just selected as the recipient of the 2018 Women in Science Award from the International Society for Antiviral Research.

Monday
June 11, 2018
12:00 PM – 1:45 PM
NOBLE HALL

6th Annual WOMEN IN SCIENCE ROUNDTABLE

This session is open to all ICAR attendees, both women and men and will feature discussions on the challenges and opportunities encountered by women scientists while navigating the twists and turns of career progression in today's environment.

Please join us to network with fellow scientists in industry, government, and academia who conduct all aspects of antiviral research. This roundtable will provide an opportunity to participate in a panel discussion with our 2018 WIS Speaker Award recipient, Dr. Angelle Desiree LaBeaud, Stanford University School of Medicine; Dr. Joana Duarte Da Rocha-Pereira, Rega Institute KU Leuven; and Dr. Heather Greenstone, NIAID, NIH, as well as other antiviral research scientists.

Registration starts at Noon with the program beginning at 12:30 PM. This event is free, but it is limited to 80 participants.

ICAR Career Development Panel

Please join us for a panel discussion about career opportunities in antiviral research at the 31st ICAR meeting. This year we will host an excellent group of panelists who are recognized experts in various areas of antiviral research and have pursued successful career in academia, government, or industry. As part of a moderated panel discussion, they will be ready to share their experience, answer questions, and provide feedback about career development. They will highlight the similarities and differences between different sectors of antiviral research. At the end of the formal panel discussion, you will have opportunity to informally network with your colleagues and panelists and make new contacts.

The event is open to all ICAR attendees. Please sign up during the ICAR conference at the registration desk before 8:30 AM on Wednesday, June 13.

The 2018 Chu Family Foundation Scholarship Awardees

ISAR and The Chu Family Foundation (TCFF) Committee are pleased to announce the winners of the 2018 Chu Family Foundation Scholarship for Women Scientists. This scholarship supports the professional development of early career level women with the potential for significant contribution in the field of antiviral research.

2018 TCFF AWARDEES

María Laura Morell

BUENOS AIRES, ARGENTINA

My name is María Laura Morell. I am a young Argentinean scientist. My passion about science started when I was a little kid. Such interest in science made me choose Biology as a career at the University of Buenos Aires (UBA). I graduated as a Molecular Biologist from UBA in 2015. That year, I was granted a Doctoral Fellowship from UBA to obtain my PhD degree. Since then I have been working as a PhD student at the Antiviral Strategies Laboratory, School of Sciences, UBA.

My doctoral thesis research focuses on the study of antiviral cellular mechanisms triggered in response against viral infection. We have demonstrated for the first time the antiviral activity of the cellular protein viperin during Junín (JUNV) arenavirus infection. Now, we would like to elucidate the cellular-viral interactions behind this antiviral mechanism, and we are currently performing colocalization assays.

I plan to assign The Chu Family Foundation award to perform an internship at Dr. Anna Överby Laboratory for Molecular Infection Medicine Sweden (MIMS), in Umeå University, Sweden. There, I will map the interactions between JUNV viral proteins and viperin, including different deleterious viperin mutants through immunoprecipitation assays. These experiments, hopefully will shed a light on viperin antiviral mechanism against arenaviruses.

Alba Torrents de la Peña

AMSTERDAM, NETHERLANDS

Alba Torrents is a PhD student at the Academic Medical Center in Amsterdam, where she works on the design of a stable HIV-1 vaccine under the supervision of Prof. Dr. Rogier Sanders. With the TCFF scholarship she wishes to have a chance to learn theoretical knowledge in vaccinology and management skills by attending the "Advanced Vaccinology" course at the Institute of Tropical Medicine and International Health in Berlin, Germany and the "Laboratory Leadership" course organized by EMBO in Heidelberg, Germany, respectively. She is highly interested in further improving her PhD research project and developing her career in vaccinology.

Crystall Swarbrick

SINGAPORE, SINGAPORE

Crystall obtained her PhD from Charles Sturt University in structural biology in 2015 and moved to Singapore to work as a postdoctoral fellow in Prof. Subhash Vasudevan's Experimental Therapeutics lab at Duke-NUS Medical School. Here she has been working toward understanding the biochemical and biophysical relationships of the dengue non-structural (NS) proteins focusing on NS3 and NS5 and their interaction with RNA and host proteins. To this end she has been working on collecting cryoEM images of complexes of NS5 with host nuclear transport proteins. She will use her TCFF award to undertake training in cryoEM techniques with Dr. Daniel Luque, who has made significant contributions to the characterization of a number of dsRNA and ssRNA viruses as well as dsDNA bacteriophages, using cryo-electron microscopy.

2018 ICAR Speaker Biographies

William Britt, MD

William Britt has focused his research on studies of viruses, ranging from retroviruses to herpesviruses. His laboratory has made fundamental contributions to current understanding of virus structure and assembly of human cytomegalovirus as well as in the definition of the antigenicity and immunogenicity of viral proteins that in some cases have been selected for vaccine development. He has also maintained a significant research effort in studies of congenital cytomegalovirus (CMV) infection which worldwide, is the most common virus associated cause of hearing loss and neurodevelopmental abnormalities. His studies of this common perinatal infection have spanned nearly 3 decades and have defined fundamental characteristics of this infection, including the paradigm shifting demonstration that normal seroimmune women can be reinfected with new strains of CMV. More recently his studies have focused on the neuropathogenesis of human CMV infections, including the use of patient cohorts and informative animal models to translate basic immunological and virological findings in these models into new therapies. He currently leads NIH supported multi-investigator studies with scientists from Brazil, Germany, Croatia, and in several centers in the US.

Thijn Brummelkamp, PhD

Thijn Brummelkamp received his Msc in biology from the Free University in Amsterdam and his PhD from Utrecht University in 2003. After his PhD he led a research group at the Whitehead Institute for Biomedical Research in Cambridge, USA. In 2011 his team moved to the Netherlands Cancer Institute in Amsterdam and he became an Adjunct PI at Center for Molecular Medicine (CeMM) in Vienna. He is a co-founder of the biotech companies Haplogen (in Vienna) and Scenic Biotech (in Amsterdam). He develops and applies genetic technologies in human cells to study biological processes. The development of application of haploid human cells as a genetic model system resulted in the identification of host factors for pathogens such as the Niemann-Pick C1 gene as the long-sought entry receptor for Ebola virus.

For his studies he received the Antoni van Leeuwenhoek Award (2003), The Annual NVBMB Award (2004, Dutch Association for Biochemistry and Molecular Biology); he was chosen as one of the world's top 35 Young Innovators by MIT's Technology Review Magazine (2005); received the Kimmel Scholar Award (2006); an ERC starting grant from the European Research Council (2012); the 2012 Molecular Biosystems Early Career Award; EMBO's Gold Medal 2013; and the Ammodo KNAW Award (2015).

Sarah Butcher, PhD

Sarah Butcher has over 25 years' experience in structural virology in academia. Currently, she is Professor of Microbiology in the Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences and Research Director in the Structural Biology and Biophysics Programme of the Institute of Biotechnology, both located in the University of Helsinki, Finland. She focuses on virus structure and assembly of emerging viruses, in their diagnostics and in potential novel antiviral therapies. She made her first advances in electron cryo microscopy and image reconstruction, in the European Molecular Biology Laboratory in Heidelberg, Germany in the '90s during her PhD and currently heads the national facility in Finland. Interested in applied research since an early age, her first degree was in Applied Biology in the University of Bath in the UK, including industrial secondments in Alko, and in Unilever. The former led her to Finland, and she is currently on her third "visit" which has now extended for 20 years! She has worked on many different viral families, (Paramyxo, Entero, Parecho, Alpha, Flavi, Pneumo, Reo as well as many bacteriophage and archaeal viruses). Most recently she has been elucidating the role of RNA in the coassembly of human parechoviruses, and its potential as a drug target.

Angela Ciuffi, PhD

Angela Ciuffi obtained her PhD in 2002 at the University of Lausanne, in Lausanne, Switzerland. She is currently a Senior Faculty at the Institute of Microbiology, which is affiliated with both the Lausanne University Hospital and the University of Lausanne. She was quickly fascinated by virology and in particular by the human immunodeficiency virus, and she thus started to work on this virus already from her Bachelor, and still does. She worked with Prof. Pascal Meylan, Prof. Frederic Bushman and Prof. Amalio Telenti, that she all considers as essential and inspiring mentors for her scientific career. Her field of expertise consists in HIV and its interaction with the host cell, leading to successful HIV replication. In particular, her research interests focus on HIV integration, HIV latency and innate immunity, fields in which she published several seminal papers. She recently started to use single-cell technologies to capture cell heterogeneity and investigate cellular differences on HIV replication, HIV latency and HIV reactivation. Dr. Ciuffi is also deeply involved in local and national activities. She is indeed a dedicated teacher at the School of Biology of the University of Lausanne, she is member of the Scientific Board of the Swiss HIV Cohort Study, and she is part of the steering and scientific committee of the Swiss Virology meeting.

Davide Corti, PhD

Davide Corti obtained his bachelor in Pharmaceutical Biotechnology at the University of Milan and his PhD in Immunology at the University of Bern, followed by postdoctoral training in Antonio Lanzavecchia's laboratory at the Institute for Research in Biomedicine where he further developed and optimized two methods for the isolation of human monoclonal antibodies out of memory B cells and plasma cells (Cellclone technologies). Since 2009 he is the Chief Scientific Officer at Humabs, where he leads a research group to isolate monoclonal antibodies against multiple infectious disease agents. Starting from 2012 he has collaborated extensively with MedImmune on the isolation of human antibodies against multiple target pathogens like Rhinovirus, Influenza B, Klebsiella and Staphylococcus. His teams' efforts to date have generated two clinical candidates, MEDI8852 targeting Influenza A and anti-CMV antibodies currently in Phase 2 studies.

Dr. Corti has published 70 peer reviewed journal articles and held 15 patents. Humabs was recently acquired by Vir Biotechnology to become its subsidiary. Humabs and its employees will continue to operate the Humabs facilities in Bellinzona, Switzerland and maintain its productive research collaboration with the Institute for Research in Biomedicine. Dr. Davide Corti will continue to be the CSO of Humabs BioMed.

David Durantel, PhD

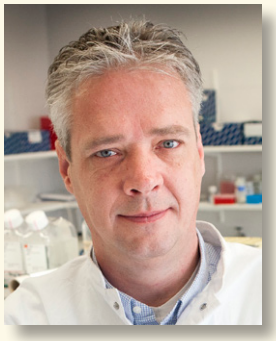
David Durantel obtained his PhD at the University of Montpellier in 1997. After three postdoctoral trainings respectively at Oxford Brookes University (UK), University of Oxford, and at INSERM-U271, he obtained a tenure position at INSERM in 2005, his *Habilitation* in 2008 from the University of Lyon (UCBL), and was recently promoted Director of Research. He currently heads a group at the Cancer Research Center of Lyon (CRCL, INSERM-U1052) in France on a program of research aiming at better understanding the interplay between HBV/HDV and liver innate immunity in order to contribute to the development of novel immune-therapeutics.

He has been involved in the past on several research projects related to drug discovery, in particular research on HCV/HBV morphogenesis inhibitors, research on PRR agonists as potential adjuvant for immune-therapeutic concepts, as well as to antiviral resistance.

He has authored/co-authored 85 PubMed-recorded publications, as well as numerous reviews/editorials, proceedings and book chapters. He acts as reviewers for many journals, including *Gastroenterology*, *Gut, Hepatology, J. Hepatol, Plos-Pathogen*, etc.

Since 2014, he is editor for the *Antiviral Research* journal, section viral hepatitis.

He contributes to national coordination on HCV/HBV/HDV research at ANRS and is a member of the executive board of AFEF (French association for liver research).



Ron Fouchier, PhD

Ron Fouchier received a PhD in Medicine from the University of Amsterdam in 1995 for studies on HIV and continued to study HIV as a post-doc at the Howard Hughes Medical Institute, University of Pennsylvania School of Medicine in Philadelphia, from 1995-1998. He subsequently started a new group to study the molecular biology of respiratory viruses, in particular influenza A virus, at the Viroscience Department of Erasmus MC Rotterdam. As a fellow of the Royal Dutch Academy of Sciences (KNAW), he studied influenza virus zoonoses and pathogenicity. Achievements of his team include the identification and characterization of several "new" viruses; the human metapneumovirus (HMPV), human coronavirus NL63, the SARS coronavirus (SARS-CoV), the MERS coronavirus (MERS-CoV), and a new influenza A virus subtype (H16). Currently, his research is focused on the evolution and molecular biology of respiratory viruses in humans and animals, with special emphasis on influenza virus antigenic drift, zoonoses, transmission, and pandemics and on HMPV. Fouchier is an elected member of the KNAW and the Royal Holland Society of Sciences and Humanities, recipient of the 2006 Heine-Medin award of the European Society for Clinical Virology and the 2013 Huibregtsen prize for top innovative science with societal impact. He co-authored more than 350 publications that have been cited >30,000 times. Fouchier is an editor for several infectious disease journals, and member of advisory committees for Dutch government and (international) scientific organizations and conferences. He is Scientific Director of the postgraduate school Molecular Medicine and the MSc program Infection and Immunity of Erasmus MC. His group is part of an NIH/NIAID Center of Excellence for Influenza Research, and his research is further funded by several EU programs and Dutch and US government.

Sir Michael Jacobs, PhD

Michael Jacobs is Consultant Physician in Infectious Diseases at the Royal Free Hospital in London. He trained at Oxford and London universities before completing a PhD in Virology. He is interested in all aspects of clinical infectious diseases with a special interest in serious viral infections. He is director of the UK High Level Isolation Unit and is a member of the UK Advisory Committee on Dangerous Pathogens. He worked at the centre of the UK response to the West Africa Ebola outbreak, and served on several national and international Ebola advisory committees. He is NHS England Programme Director for High Consequence Infectious Diseases. He was knighted in 2016 for services to the prevention and treatment of infectious diseases.

David Kimberlin, MD

David Kimberlin holds the Sergio Stagno Endowed Chair in Pediatric Infectious Diseases at the University of Alabama at Birmingham, where he is Vice Chair for Clinical and Translational Research and Co-Director of the Division of Pediatric Infectious Diseases. Dr. Kimberlin also is the Principal Investigator for the Collaborative Antiviral Study Group (CASG). Funded continuously by NIH/NIAID/DMID since the early 1970s, the CASG is a network of pediatric academic medical centers that evaluates antiviral therapeutics in rare diseases with a large unmet medical need, including neonatal herpes simplex virus (HSV) infections, congenital cytomegalovirus (CMV) disease, congenital Zika syndrome, neonatal and infantile influenza infection, and neonatal enteroviral sepsis syndrome. The number of participating academic medical centers varies by study, but generally ranges from 15 to 30 across the United States, the United Kingdom, and Peru. Studies conducted by the CASG have led to new drug indications and label changes for acyclovir, valganciclovir, and oseltamivir, and non-CASG studies conducted by Dr. Kimberlin also have led to label changes for valacyclovir. Current CASG studies are evaluating novel diagnostic modalities for and incidence of neonatal herpes, treatment studies of congenital CMV infections, and natural history studies of Zika virus infection in pregnancy. These studies build upon previous CASG studies conducted by Dr. Kimberlin that have defined the standard of care for the treatment of neonatal HSV and congenital CMV infections.

Dr. Kimberlin also is Editor of the 2018 AAP *Report of the Committee on Infectious Diseases (Red Book)*, and was Editor of the 2015 edition and Associate Editor for the 2009 and 2012 editions. He served as a member of the American Academy of Pediatrics (AAP) Committee on Infectious Diseases (COID) from 2005-2011. Dr. Kimberlin also is the AAP Red Book liaison to the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP), having served in this capacity since 2006. During this time, he has served as the COID liaison to six different ACIP Working Groups.

Dr. Kimberlin is a Past-President of the Pediatric Infectious Diseases Society (PIDS), which is the world's largest organization of professionals dedicated to the treatment, control, and eradication of infectious diseases affecting children. In 2016 he received the Ronald McDonald House Charities 2016 Medical Award of Excellence. He has received numerous education awards.

Percy Knolle, MD

Percy Knolle is currently the director of the Institute of Molecular Immunology and Experimental Oncology at the University Hospital München rechts der Isar of the Technical University Munich, Germany.

Prof. Knolle studied medicine at the Universities of Frankfurt, Paris, Birmingham (UK), Strasbourg, and Geneva and completed his MD thesis at the German Cancer Research Center (Applied Immunology, Prof. Meuer) in 1990 in Heidelberg. After a postdoctoral fellowship with the Bioresearch Corporation of BASF Cambridge (USA) he joined the 1st Medical Department at University Hospital in Mainz (Prof. Meyer zum Büschenfelde) in 1991 where he completed his training and qualification as specialist in Internal Medicine in 1997. From 1997 until 2002, Prof. Knolle held the position of an independent research group leader at the Center of Molecular Biology in Heidelberg, Germany (ZMBH), where he received a Volkswagenstiftung Research Award.

In 2002 he was appointed full professor at the University of Bonn (Germany). He was founding director of the Institutes of Molecular Medicine and Experimental Immunology. In 2013, Prof. Knolle accepted a full professorship at the Faculty of Medicine and the Faculty of Natural Sciences at the Science Campus Weihenstephan, Technical University of Munich.

Randi Leavitt, MD, PhD

Randi Leavitt received her Medical and PhD degree from Washington University in St. Louis in 1978. She completed her Internal Medicine training at Yale-New Haven Hospital. She spent the next 11 years in the Laboratory of Immunoregulation NIAID where she did fellowship training in infectious diseases and allergy/immunology and was subsequently a Senior Investigator in the Laboratory of Immunoregulation NIAID. Dr. Leavitt has specialty boards in Internal Medicine, Infectious Diseases and Allergy and Immunology. Dr. Leavitt joined Merck in 1993 and has been involved with clinical studies of antiviral drugs including CRIXIVAN™, Stocrin, Isentress and letermovir and HIV Vaccine development. Presently, Dr. Leavitt is an Executive Director, Infectious Diseases at Merck & Co., Inc.

Philippe Lemey, PhD

Philippe Lemey is an Associate Professor at the Rega Institute, University of Leuven. His research interests lie in the fields of molecular epidemiology, computational biology and viral evolution. In particular, he has studied the evolutionary processes that shape viral genetic diversity, spanning from large-scale epidemic processes, such as population growth and spatial dispersal, to small-scale transmission histories and within-host evolutionary processes. Specific applications include the origin of HIV, the source-sink dynamics of seasonal influenza, rabies metapopulation dynamics, and Ebola virus patterns of spread. Philippe is the lead author of the second edition of the Phylogenetic Handbook and a two-time ERC grant awardee. His team has made important contributions to the popular BEAST software, which was acknowledged by the Mitchell prize in Bayesian statistics.

Nicolas Manel, PhD

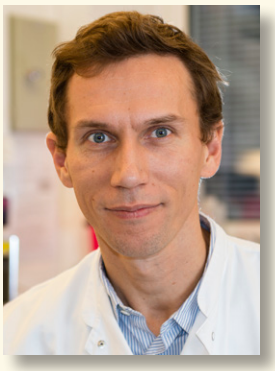
Nicolas Manel is senior group leader at Institut Curie, Paris, France and holds a position of Director of Research at INSERM. He obtained his PhD from University of Montpellier, France, where he identified the entry receptor for HTLV in the lab of Marc Sitbon. He performed his postdoctoral training in the lab of Dan Littman at New York University School of Medicine. There, he demonstrated that HIV-1 escapes from an immune response in dendritic cells. He also investigated CD4+ Th17 lymphocytes of humans and mice. Since 2010, his lab at Institut Curie is interested in the basic principles that operate at the intersection between innate immunity and viral replication, and their impact on adaptive immunity, focusing on the HIV model. His lab identified cGAS as an essential sensor of HIV in dendritic cells. The lab also discovered a Trojan horse mechanism of immune signal transmission, based on the transfer of the immune second messenger cGAMP by viral particles between cells. The lab recently showed that the nuclear envelope protein SUN2 modulates early steps of HIV replication. Currently, the lab is exploring the principles that allow immune cells to recognize and manage viral infections.

Jean-Michel Pawlotsky, MD, PhD

Jean-Michel Pawlotsky is Professor of Medicine at the University of Paris-Est. He is the Director of the National Reference Center for Viral Hepatitis B, C and D and of the Department of Virology at the Henri Mondor University Hospital in Créteil, France, and Director of the Academic Department "Viruses, Immunity and Cancers"(INSERM U955). He focuses on teaching and research in virology (primarily hepatitis viruses) and liver oncology. Dr. Pawlotsky earned his medical degree in Hepatology and Gastroenterology in 1992. In addition, he earned a Thesis in molecular virology from the University of Paris, France, and he is a graduate in virology from the Pasteur Institute in Paris and microbiology from the University of Paris. Dr. Pawlotsky is active in numerous professional societies, and has been acting as the Secretary General of the European Association for the Study of the Liver (EASL) between 2005 and 2009. He is the President of Scientific Commission 4 (CSS4) and Concerted Action 33 (AC33) of the French National Agency for Research on AIDS and Viral Hepatitis (ANRS). Dr. Pawlotsky has been an Associate Editor of *Hepatology*, the official journal of the American Association for the Study of Liver Diseases (AASLD), between 2001 and 2006, and an Associate Editor of *Gastroenterology*, the official journal of the American Gastroenterological Association (AGA), between 2011 and 2016. He is a member of the Editorial Board of the *Journal of Hepatology*, *Therapeutic Advances in Gastroenterology*, and *European Gastroenterology and Hepatology Review*. Dr. Pawlotsky's noted career contributions include the publication of over 500 articles and book chapters in his areas of expertise and over 600 invited lectures at international meetings.

Xavier Saelens, PhD

Xavier Saelens obtained his PhD in the laboratory of Walter Fiers at Ghent University. He is currently a group leader in the VIB-UGent Center for Medical Biotechnology and a full professor at Ghent University (Belgium). The research of his group is primarily focused on the development of novel vaccines and antibody-based antivirals against influenza A and B viruses and human Respiratory Syncytial virus. A more fundamental research line of his team is to try to elucidate how the interferon induced GTPase MX1 exerts its antiviral activity. His group is well known for its work on a broadly protective influenza vaccine that is based on M2e, an approach that was conceived by Walter Fiers. More recently, his group proposed a novel human RSV vaccine candidate and reported the discovery of prefusion F-specific single domain antibodies that can potently neutralize RSV.



Ole Schmeltz Søgaard, MD, PhD

Søgaard is a MD, PhD at the Department of Infectious Diseases at Aarhus University Hospital, Denmark. As a physician-scientist with training both in basic science immunology/virology and clinical medicine, his research has mainly been focused on the understanding of HIV-1 pathogenesis and persistence, including clinical investigations into immune modulation therapies and reversal of HIV-1 latency. As principal investigator Dr. Søgaard has lead several investigator-initiated trials investigating the effects of the latency-reversing agent romidepsin with and without therapeutic HIV vaccination as well as the use of TLR9 agonists as adjunctive therapy. He was also co-investigator on the panobinostat study. In 2016, Dr. Søgaard spent a sabbatical year as visiting associate professor at Rockefeller University and works with professor Michel Nussenzweig in the development of antibody based HIV curative strategies. Dr. Søgaard is co-director of the Danish clinical HIV latency and cure research

Frank van Kuppeveld, PhD

Frank van Kuppeveld obtained his PhD in the lab of Medical Microbiology at the University of Nijmegen, The Netherlands. The research of his group primarily focused on picornaviruses (e.g. enteroviruses, rhinoviruses, EMCV and FMDV), with particular interest in identifying receptors, entry mechanisms, and the molecular aspects of viral genome replication. Using state-of-the-art virological, biochemical, cell biological (e.g. siRNA and haploid genetic screens), and microscopy (e.g. life cell imaging and tomography) approaches, his group extensively studies the role of *picornavirus* proteins and hijacked host factors in the formation and the architecture of the viral replication organelles as well as in the process of viral RNA synthesis that takes place in these specialized replication sites. In 2012, he moved with his whole group to the University of Utrecht to become chair and full professor at the Virology department (which has a longstanding tradition on basic and translational research on influenza and coronaviruses). His work has been rewarded with prestigious prizes from the Royal Netherlands Academy of Sciences and grants of the Netherland Organization of Scientific Research (e.g. VIDI and VICI grants). He coordinated the EU Training Network "EUVIRNA" on +RNA virus replication and antiviral drug development (2011-2015) and is currently coordinating the EU Training Network "ANTIVIRALS" (2015-2019). group.

Marco Vignuzzi, PhD

Marco Vignuzzi has been a faculty member at Institut Pasteur since 2008, developing computational and experimental tools to study RNA virus evolution. His laboratory studies the behavior of viruses as populations during infection, trying to identify stages of the virus multiplication, dissemination and transmission cycle that are most sensitive to antiviral strategies. His lab takes an evolutionary perspective to target virus fitness by trying to hinder, alter or subjugate their adaptation and evolution.

Annelies Wilder-Smith, MD, PhD

Annelies Wilder-Smith is Consultant to the World Health Organization on arboviral diseases, and part-time Professor of Emerging Infectious Diseases at the London School of Hygiene and Tropical Medicine and the Lee Kong Chian School of Medicine, Singapore. She is the Principal Investigator of the EU funded international consortium "ZikaPLAN" (*https://zikaplan.tghn.org/)* funded by the European Commission under Horizon 2020 overseeing 15 work packages and 25 institutional global partners to address research gaps related to Zika and to set up a Latin American research preparedness network for emerging diseases. From 2011 to 2016, she led another EU funded research consortium, called DengueTools, to work on innovative tools for the surveillance and control of dengue.

A physician with expertise in travel and tropical medicine, she is the Immediate Past President of the International Society of Travel Medicine (ISTM), Past-President of the Asia Pacific Society of Travel Medicine. Her special research interests include vaccine preventable and emerging infectious diseases, in particular related to arboviral diseases. With a career spanning almost three decades, she has led and co-led various clinical trials, published more than 240 scientific papers, edited and co-edited textbooks, and serves on various editorial boards and scientific committees. Her awards include the Myrone Levine Vaccinology Prize, the Honor Award for exemplary leadership and coordination in determining and communicating global yellow fever risk presented at the CDC Award Ceremony, the Mercator Professorship award by the German Research Foundation and the Ashdown Oration Award by the Australian College of Travel Medicine.

Program Schedule

MONDAY, JUNE 11th, 2018

Satellite Workshop of the EU H2020 Consortium 'ANTIVIRALS'

Chair: **Frank van Kuppeveld**

ARCHIVE HALL I

2:00 PM – 3:45 PM

(Abstract Number)

2:00 PM **242.** **ANTIVIRALS, a European Training Network to Train the Next Generation of Experts in Antiviral Drug Development**
Frank van Kuppeveld, PhD
Virology Division, Dept. of Infectious Diseases & Immunology, Utrecht University, Utrecht, The Netherlands

2:30 PM **13-14 ESRs Shotgun Presentation Session**

3:00 PM **How an Old Dog Performs New Tricks: Mechanistic Insight into the Mode of Action of Fluoxetine Targeting Enterovirus 2C^{ATPase}**
Lisa Bauer
Virology Division, Faculty of Veterinary Medicine, Utrecht University, the Netherlands

3:15 PM **16.** **Heteroarylpyrimidine (HAP) and Novel Non-HAP Capsid Assembly Modifiers Show Differences in their Mode of Action *In Vitro***
Angelica Corcuera
AiCuris Anti-Infective Cures GmbH, Wuppertal, Germany

Welcome/Keynote Addresses and Recent Technological Advances

Chairs: **Andrea Brancale** and **Johan Neyts**

ARCHIVE HALL I

3:45 PM – 6:45 PM

3:45 PM **245.** **Hepatitis C Virus: Problem Solved. And Now?**
Jean-Michel Pawlotsky, MD, PhD
University of Paris-Est, Paris, France

4:30 PM **231.** **Mutagenesis of the Human Genome to Study Virus Entry**
Thijn Brummelkamp, PhD
Netherlands Cancer Institute, Dept. of Biochemistry, Amsterdam, The Netherlands

5:30 PM **232.** **A Primer on Cryo-EM and Image Reconstruction for Antiviral Drug Development**
Sarah Butcher, PhD
University of Helsinki, Helsinki, Finland

6:00 PM **244.** **Epidemic Arboviral Diseases: Implications for Research and Public Health Infrastructure**
Annelies Wilder-Smith, MD, PhD
Umeä University Sweden and WHO, Geneva, Umeä, Sweden

Coffee Break
RIBEIRA I & II
5:00 PM – 5:30 PM

Opening Reception
NOBLE HALL – GROUND FLOOR
6:45 PM – 8:00 PM

TUESDAY, JUNE 12th, 2018

Women in Science Award Lecture
ARCHIVE HALL I
8:30 AM – 9:00 AM

Making the Invisible Visible: Arbovirus Transmission, Risk, Disease and Prevention in Kenya
A. Desiree LaBeaud, MD, MS
Pediatric Infectious Diseases, Stanford University, Stanford, CA, United States of America

Virus Evolution Session
Chair: **Johan Neyts**
ARCHIVE HALL I
9:00 AM – 10:00 AM

9:00 AM **238.** **Getting to the Root of Epidemic Spread: An Evolutionary Perspective on Pathogen Emergence**
Philippe Lemey, PhD
Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium

9:30 AM **246.** **Monitoring, Predicting and Altering the Evolution of RNA Virus Populations**
Marco Vignuzzi, PhD
Institut Pasteur, Paris, France

Coffee Break
RIBEIRA I & II
10:00 AM – 10:30 AM

Emerging Infections Oral Session

Chairs: **Leen Delang** and **Marcella Bassetto**

ARCHIVE HALL I

10:30 AM – 12:15 PM

10:30 AM **235. Ebola Treatment: Working in the Dark under the Spotlight**

Sir Michael Jacobs, PhD

Royal Free London NHS Foundation Trust, London, United Kingdom

11:00 AM **206. Alpha-Ketoamides as Broad-Spectrum Inhibitors of Coronavirus and Enterovirus Replication**

Rolf Hilgenfeld, Ph.D.[1], Daizong Lin, Ph.D.[1], Yuri Kusov, Ph.D.[2], Qingjun Ma, Ph.D.[2], Albrecht von Brunn, Ph.D.[3], Pieter Leyssen, Ph.D.[4], Kristina Lanko, Ph.D.[4], Johan Neyts, Ph.D.[4], Adriaan de Wilde, Ph.D.[5], Eric Snijder, Ph.D.[5], Hong Liu, Ph.D.[6], Linlin Zhang, Ph.D.[1]

[1]Institute of Biochemistry & German Center for Infection Research (DZIF), University of Luebeck, Luebeck, Germany; [2]Institute of Biochemistry, University of Luebeck, Luebeck, Germany; [3]Max von Pettenkofer Institute, Ludwigs-Maximilian University Munich, Munich, Germany; [4]Rega Institute, University of Leuven, Leuven, Belgium; [5]University of Leiden Medical Center, Leiden, Netherlands; [6]Shanghai Institute of Materia Medica, Shanghai, China

11:10 AM **210. Therapeutic Treatment of Zika Virus Infection Using a Brain-Penetrating Antiviral Peptide**

Nam-Joon Cho, Ph.D.

Materials Science and Engineering Nanyang Technological University, Singapore, Singapore

11:20 AM **196. Reverse Genetics Identifies Chikungunya Virus nsP1 as the Target for a Novel Nucleoside Analogue**

Kristina Kovacikova, B.S.[1], Ali Tas, B.S.[1], Irina Albulescu, M.S.[1], Jinha Yu, Ph.D.[2], Jarhad Dnyandev, Ph.D.[2], Gyudong Kim, Ph.D.[2], Pramod Sahu, Ph.D.[2], Lak Shin Jeong, Ph.D.[2], Martijn van Hemert, Ph.D.[1]

[1]Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands; [2]Laboratory of Medicinal Chemistry, College of Pharmacy, Seoul National University, South Korea

11:30 AM **202. Strategies of Tick-Borne Encephalitis Virus to Escape from Nucleoside Analogue-Mediated Inhibition**

Ludek Eyer, Ph.D.[1], Daniel Ruzek, Ph.D.[1]

[1]Department of Virology, Veterinary Research Institute, Brno, Czechia

11:40 AM **214. Role of Nonstructural Protein 1 in the Replication Cycle of Dengue Virus**

Anna Płaszczyca, M.S.[1], Pietro Scaturro, Ph.D.[2], Christopher Neufeldt, Ph.D.[3], Ralf Bartenschlager, Ph.D.[1]

[1]Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany

11:50 AM **216. *In Vivo* Replication of Human Norovirus in Zebrafish Larvae**

Jana Van Dycke, M.S.[1], Annelii Ny, Ph.D.[2], Nádia Conceição-Neto, Ph.D.[3], Jan Maes, B.S.[2], Myra Hosmillo, Ph.D.[4], Ian Goodfellow, Ph.D.[4], Tatiane C. Nogueira, Ph.D.[1], Charlotte Vanderheydt, M.S.[1], Jelle Matthijnssens, Ph.D.[3], Peter de Witte, Ph.D.[2], Johan Neyts, Ph.D.[1], Joana Rocha-Pereira, Ph.D.[1]

[1]KU Leuven – Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium, Leuven, Belgium; [2]KU Leuven – Laboratory for Molecular Biodiscovery, Leuven, Belgium., Leuven, Belgium; [3]KU Leuven – Rega Institute, Laboratory of Clinical and Epidemiological Virology, Leuven, Belgium, Leuven, Belgium; [4]University of Cambridge, Division of Virology, Addenbrooke's Hospital, Cambridge, CB2 0QQ, United Kingdom., Cambridge, United Kingdom of Great Britain and Northern Ireland

Lunch

NOBLE HALL

12:15 PM – 1:30 PM

(Various options available for purchase)

Influenza Virus Session

Chairs: **Amy Krafft** and **Brett Hurst**

ARCHIVE HALL I

1:30 PM – 2:35 PM

1:30 PM **247. Passive Antibody Therapies Against Endemic, Emerging and Re-Emerging Viral Infectious Diseases**

Davide Corti, PhD

Humabs BioMed, a subsidiary of Vir Biotechnology, Bellinzona, Switzerland

2:00 PM **243. Influenza Virus Infection, Neuraminidase Inhibitor Treatment, and Emergence of Drug Resistance in an Immunocompromised Ferret Model**

Ron Fouchier, PhD

Department of Viroscience, Erasmus MC, Rotterdam, The Netherlands

Coffee Break

RIBEIRA I & II

2:35 PM – 3:15 PM

Respiratory Viruses Session

Chairs: **Amy Krafft** and **Brett Hurst**

ARCHIVE HALL I

3:15 PM – 5:00 PM

3:15 PM **240. Treatment Options for Human RSV: Small Molecules, Antibodies or Something in Between?**

Xavier Saelens, PhD

VIB Center for Medical Biotechnology, Department for Biomedical Molecular Biology, Ghent University, Ghent, Belgium

3:40 PM **220. Differential Antiviral Activities of RSV Inhibitors in Human Airway Epithelium**

Carmen Mirabelli, Ph.D.[1], Martine Jaspers, Ph.D.[1], Mieke Boon, M.D., Ph.D.[1], Mark Jorissen, M.D., Ph.D.[2], Mohamed Koukni, Ph.D.[3], Dorothee Bardiot, Ph.D.[3], Patrick Chaltin, Ph.D.[1], Arnaud Marchand, Ph.D.[3], Johan Neyts, Ph.D.[1], Dirk Jochmans, Ph.D.[4]

[1]KU Leuven, Leuven, Belgium; [2]UZ Leuven, Leuven, Belgium; [3]CISTIM, Leuven, Belgium; [4]KU Leuven, Rega Institute, Virology and Chemotherapy, Leuven, Belgium

3:55 PM **197. Treatment of an EV-D68 Infection with Human Intravenous Immunoglobulin (hIVIG) in a Respiratory and Neurological Model in AG129 Mice.**

Brett Hurst, M.S.[1], William Evans, M.S.[1], Christopher Peterson, B.S.[1], Donald Smee, Ph.D.[1], Bart Tarbet, Ph.D.[1]

[1]Utah State University, Logan, Utah, United States of America

4:10 PM **227. Antiviral and Immunological Adjuvant Efficacy of Synthetic RNA as a RIG-I Agonist against Influenza A Virus Infection**

Meehyein Kim, Ph.D.[1], Janghyun Lee, Ph.D.[2], Yun Young Go, Ph.D.[1], Jin Soo Shin, D.V.M.[1], Chong-Kyo Lee, Ph.D.[3], Suk-Jo Kang, Ph.D.[4], Byong-Seok Choi, Ph.D.[5]

[1]Virus Research Group, Korea Research Institute of Chemical Technology (KRICT), Daejeon 34114, Republic of Korea; [2]Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, South Korea; [3]Convergent Research of Emerging Virus Infection, KRICT, Daejeon 34114, Republic of Korea; [4]Department of Biological Sciences, KAIST, Daejeon 34141, South Korea; [5]Department of Chemistry, KAIST, Daejeon 34141, South Korea

4:25 PM **49. The Host-Targeted Iminosugar UV-4B Inhibits Influenza Virus without Selecting for Resistance**

Lisa Evans DeWald, Ph.D.[1], Urban Ramstedt, Ph.D.[2], Dale Barnard, Ph.D.[3], Michelle Mendenhall, Ph.D.[3], Eric Stavale, M.S.[4], Suman Das, Ph.D.[2], Kelly Warfield, Ph.D.[1]

[1]Emergent BioSolutions; [2]J. Craig Venter Institute; [3]Utah State University; [4]Integrated BioTherapeutics

Poster Session 1

ARCHIVE HALL II

5:00 PM – 7:00 PM

5:00 – 6:00 PM **ODD numbered poster presentations**

6:00 – 7:00 PM **EVEN numbered poster presentations**

All posters are listed in numerical order starting on page 36.

WEDNESDAY, JUNE 13th, 2018

Antonín Holý Memorial Award Lecture

ARCHIVE HALL I

8:30 AM – 9:00 AM

From Nucleoside Monophosphate Prodrugs to Nucleoside Triphosphate Prodrugs – The Challenge, a Possible Solution and Further Improvements

Chris Meier, PhD

University of Hamburg, Hamburg, Germany

Antiviral Research: Annual Journal Summary

Chair: **Mike Bray**

ARCHIVE HALL I

9:00 AM – 9:10 AM

Viral Hepatitis Session

Chair: **Mike Bray**

ARCHIVE HALL I

9:10 AM – 10:00 AM

9:10 AM **234.** **HBc and CAMs: a Tale of "Swiss-Knife" Protein and Antivirals**

David Durantel, PhD

Cancer Research Centre of Lyon (CRCL), INSERM, U1052, UMR_5286 CNRS/University of Lyon, Lyon, France

9:35 AM **248.** **Mechanisms of Immune Dysfunction in Chronic Hepatitis B and Possible Immune Therapies**

Percy Knolle, MD

Institute of Molecular Immunology and Experimental Oncology, Technische Universitaet Muenchen, Muenchen,Germany

Coffee Break

RIBEIRA I & II

10:00 AM – 10:30 AM

Hepatitis and Retroviruses Session

Chair: **Tim Block**

ARCHIVE HALL I

10:30 AM – 12:15 PM

10:30 AM **225.** **Pharmacological Inhibition of CDK4/6 Enhances Antiviral and Cytotoxic Activity of Antimetabolites**

Marc Castellvi, M.S.[1], Eudald Felip, M.D.[2], Maria Pujantell, M.S.[1], Edurne Garcia-Vidal, M.S.[1], Bonaventura Clotet, M.D., Ph.D.[1], **Eva Riveira-Muñoz, Ph.D.**[1], Roger Badia, Ph.D.[1], Mireia Margelí, M.D., Ph.D.[2], José Esté, Ph.D.[1], Ester Ballana, Ph.D.[1]

[1]Irsicaixa AIDS Research Institute, Badalona, Spain; [2]Catalan Institute of Oncology (ICO), Spain

10:45 AM **221. ADAR1 is a Regulator of Innate and Antiviral Immune Function in HCV Infection**

Eva Riveira-Muñoz, Ph.D.[1], **María Pujantell, B.S.**[1], Sandra Franco, Ph.D.[1], Marc Castellví, B.S.[1], Edurne García-Vidal, B.S.[1], Roger Badia, Ph.D.[1], Cristina Tural, M.D., Ph.D.[2], Bonaventura Clotet, M.D., Ph.D.[1], Miguel Angel Martinez, Ph.D.[1], José Esté, Ph.D.[1], Ester Ballana, Ph.D.[1]

[1]IrsiCaixa AIDS Research Institute, Badalona, Spain; [2]Hospital Universitari Germans Trias i Pujol

11:00 AM **201. The NEDD8-Activating Enzyme Inhibitor MLN4924 Potently Inhibits Transcription from Covalently Closed Circular DNA in a Hepatitis B Virus X Protein-Dependent Manner**

Bingqian Qu, M.S.[1], Mila Leuthold, Ph.D.[1], Pascal Mutz, M.S.[2], Firat Nebioglu, M.S.[2], Ralf Bartenschlager, Ph.D.[1], Stephan Urban, Ph.D.[3]

[1]Molecular Virology, Department of Infectious Diseases, University Hospital Heidelberg, Heidelberg, Germany; [2]F170 "Virus-associated carcinogenesis" German Cancer Research Center; [3]German Centre for Infection Research (DZIF), Partner Site Heidelberg, Germany, Heidelberg, Germany

11:15 AM **223. Hepatitis B Virus Replication Inhibition by *N*-hydroxypyridinediones and N-hydroxyisoquinolinediones in Culture through Inhibition of the Viral Ribonuclease H**

Tiffany Edwards, M.S.[1], Erofili Giannakopoulou, M.S.[2], Vasiliki Pardali, M.S.[2], Grigoris Zoidis, Ph.D.[2], Marvin Meyers, Ph.D.[1], Nagraj Mani, Ph.D.[3], Bruce Dorsey, Ph.D.[3], Ramesh Kakarla, Ph.D.[3], Rene Rijnbrand, Ph.D.[3], Mike Sofia, Ph.D.[3], John Tavis, Ph.D.[1]

[1]Saint Louis University, St. Louis, Missouri, United States of America; [2]University of Athens; [3]Arbutus Biopharma Inc.

11:30 AM **200. Small Molecule-induced Degradation of Viral Targets**

Melissanne de Wispelaere, Ph.D.[1], Guangyan Du, Ph.D.[2], Tinghu Zhang, Ph.D.[2], Nathanael Gray, Ph.D.[2], Priscilla Yang, Ph.D.[1]

[1]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, United States of America; [2]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States of America

11:45 AM **222. A Novel Benzamide Compound that Distinctly Modulates HBV Capsid Assembly**

Xuexiang Zhang, M.S.[1], Junjun Cheng, Ph.D.[1], Zhanying Hu, Ph.D.[1], Qiong Zhao, Ph.D.[1], Julia Ma, B.S.[1], Shuo Wu, Ph.D.[1], Yanming Du, Ph.D.[1], Ju-Tao Guo, M.D.[1], Jinhong Chang, M.D., Ph.D.[1]

[1]Baruch S. Blumberg Institute

New Member and First Time Attendee Networking featuring a PechaKucha Competition

INFANTE HALL

12:30 PM – 1:30 PM

Career Development Panel

INFANTE HALL

1:45 PM – 2:45 PM

THURSDAY, JUNE 14, 2018

Gertrude Elon Memorial Award Lecture

ARCHIVE HALL I

8:15 AM – 9:00 AM

Quantitative Studies of HCMV in a Human Challenge Model

Paul Griffiths, MD

Centre for Virology, University College London Medical School, London, United Kingdom

Cytomegalovirus Session

Chair: **Dana Wolf**

ARCHIVE HALL I

9:00 AM – 10:30 AM

9:00 AM **230.** **Neurodevelopmental Sequelae Following Congenital CMV: Role of Virus-Induced Inflammation**

William Britt, MD

Departments of Pediatrics, Microbiology, and Neurobiology; School of Medicine, University of Alabama, Birmingham, Alabama, United States of America

9:25 AM **236.** **Treatment of Congenital CMV Infection: New Populations, New Regimens, and New Drugs?**

David Kimberlin, MD

University of Alabama, Birmingham, Alabama, United States of America

9:45 AM **237.** **Letermovir: Current State of the Art**

Randi Leavitt, MD, PhD

Clinical Research, MSD, North Wales, Pennsylvania,United States of America

Coffee Break

RIBEIRA I & II

10:30 AM – 11:00 AM

Medicinal Chemistry Session

Chairs: **Chris Meier** and **Zlatko Janeba**

ARCHIVE HALL I

11:00 AM – 12:15 PM

11:00 AM **215.** **Synthesis and Evaluation of Mono-, Di-, and Tetra-acylated Prodrugs of IHVR-19029**

Nicky Hwang, B.S.[1], Jia Guo, Ph.D.[1], Julia Ma, B.S.[1], Xuexiang Zhang, M.S.[1], Qing Su, Ph.D.[1], Timothy Block, Ph.D.[1], Jinhong Chang, M.D., Ph.D.[1], Yanming Du, Ph.D.[1]

[1]Baruch S. Blumberg Institute

11:12 AM **207.** **A Novel Chemotype as Hepatitis B Virus Capsid Assembly Effectors**

Jing Tang, Ph.D.[1], Andrew Huber, Ph.D.[2], Jayakanth Kankanala, Ph.D.[1], Eleftherios Michailidis, Ph.D.[2], Karen Kirby, Ph.D.[3], Jiashu Xie, Ph.D.[1], Stefan Sarafianos, Ph.D.[3], **Zhengqiang Wang, Ph.D.**[1]

[1]University of Minnesota; [2]University of Missouri; [3]University of Missouri, Emory University

11:24 AM **224. Mimicking the HRB Region of RSV F Protein as Antiviral Strategy**

Roberto Manganaro, M.S.[1], Dirk Jochmans, Ph.D.[2], Johan Neyts, Ph.D.[2], Pieter Leyssen, Ph.D.[2], Andrea Brancale, Ph.D.[1]

[1]Cardiff School of Pharmacy and Pharmaceutical Sciences, Cardiff, United Kingdom of Great Britain and Northern Ireland; [2]KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute, Leuven, Belgium

11:36 AM **195. Discovery of Novel Small-molecule Inhibitors Against Chikungunya Virus: Virtual Screening, Organic Synthesis and Cell-Based Mode of Action Studies**

Birgit Zonsics, M.S.[1], Rana Abdelnabi, Ph.D.[2], Leen Delang, Ph.D.[2], Ana-Sofia Ferreira-Ramos, M.S.[3], Johan Neyts, Ph.D.[2], Bruno Coutard, Ph.D.[3], Andrea Brancale, Ph.D.[4]

[1]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, Wales, United Kingdom, Cardiff, Wales, United Kingdom of Great Britain and Northern Ireland; [2]KU Leuven, Rega Institute for Medical Research, Leuven, Belgium; [3]Aix Marseille Univ, CNRS, AFMB UMR7257, Marseille, France; [4]Cardiff University, Cardiff, United Kingdom of Great Britain and Northern Ireland

11:48 AM **204. Rational Design and Synthesis of Novel Potential Inhibitors of Zika Virus Replication**

Marcella Bassetto, Ph.D.[1], Martina Salerno, M.S.[1], Juliane Nolte, M.S.[2], Benno Schreiner, M.S.[2], Joachim Bugert, M.D., Ph.D.[2], Etienne Decroly, Ph.D.[3], Bruno Coutard, Ph.D.[3], Kristina Kovacikova, M.S.[4], Martijn van Hemert, Ph.D.[4], Andrea Brancale, Ph.D.[1]

[1]Cardiff University; [2]Institut für Mikrobiologie der Bundeswehr; [3]AFMB, Aix Marseille University; [4]Leiden University Medical Center

12:00 PM **209. Gamma-Non-Symmetrically-Modified d4T Triphosphates as Anti-HIV Prodrugs**

Chenglong Zhao, M.S.[1], Chris Meier, Ph.D.[1], Yara Angeloni, M.S.[2]

[1]University of Hamburg, Hamburg, Germany; [2]University of Camerino

ISAR Business Meeting

12:15 PM – 12:30 PM

President: **José Esté, PhD**

Treasurer: **Brian Gowen, PhD**

Secretary: **Graciela Andrei, PhD**

Lunch

NOBLE HALL

12:30 PM – 1:30 PM

(Various options available for purchase)

William Prusoff Young Investigator Award Lecture

ARCHIVE HALL I

1:30 PM – 2:00 PM

Regulation of Nucleotide Metabolism: From Virus Restriction to Therapeutic Implications in HIV infection and Beyond

Ester Ballana, PhD

IrsiCaixa - Institute for AIDS Research, Badalona, Spain

HIV Treatment and Cure Session

Chair: **José Esté**

ARCHIVE HALL I

2:00 PM – 3:45 PM

2:00 PM **233. Transcriptional Profiling of HIV Latency**

Angela Ciuffi, PhD

Institute of Microbiology, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

2:25 PM **239. Identification of a Capsid-Binding Protein that Recognizes the Nuclear HIV Capsid to Promote CGAS-Mediated Innate Immune Activation in Dendritic Cells**

Nicolas Manel, PhD

Institut Curie, PSL Research University, Paris, France

2:45 PM **241. Strategies for an HIV Cure: Early Lessons from Shock and Kill Trials**

Ole Schmeltz Søgaard, MD, PhD

Dept. of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark

Poster Session 2

ARCHIVE HALL II

3:45 PM – 5:45 PM

3:45 – 4:45 PM **EVEN numbered poster presentations**

4:45 – 5:45 PM **ODD numbered poster presentations**

All posters are listed in numerical order starting on page 36.

Closing Reception

Ferreira Cellars

7:00 PM – 10:30 PM

**Advance purchase of banquet ticket required. Transportation provided from select hotels.*

FRIDAY, JUNE 15TH, 2018

Shotgun Poster Presentations

Chair: **Justin Julander**

ARCHIVE HALL I

8:30 AM – 9:00 AM

Emerging Infections and Clinical Evaluation of Antivirals Session

Chairs: **Brian Gowen** and **Jessica Spengler**

ARCHIVE HALL I

9:00 AM – 10:30 AM

9:00 AM **226.** **Chemical Genetic Studies Revealed a Novel Mechanism of Innate Immune Evasion by Yellow Fever Virus NS4B That Can Be Targeted by an Antiviral Compound**

Xuexiang Zhang, M.S.[1], Shuo Wu, Ph.D.[1], Julia Ma, B.S.[1], Fang Guo, M.D., Ph.D.[1], Justin Julander, Ph.D.[2], Timothy Block, Ph.D.[1], Yanming Du, Ph.D.[1], Ju-Tao Guo, M.D.[1], **Jinhong Chang, M.D., Ph.D.**[1]

[1]Baruch S. Blumberg Institute; [2]Utah Sate University

9:10 AM **211.** **Identification of a Novel Small Molecule Inhibitor of Lassa Fever Virus Entry that Targets the Viral Receptor, LAMP1**

May Wang, B.S.[1], Sun-Young Lim, B.S.[1], Tao Ren, Ph.D.[2], Hu Liu, Ph.D.[3], Kyungae Lee, Ph.D.[4], Anna Honko, Ph.D.[5], James Cunningham, M.D.[1]

[1]Harvard Medical School; Brigham and Women's Hospital, Boston, Massachusetts, United States of America; [2]CAS (Hefei) Institute of Technology Innovation; [3]Harvard Medical School, Boston, Massachusetts, United States of America; [4]Broad Institute; [5]Data Sciences International

9:20 AM **205.** **Labyrinthopeptin A1 Exerts Broad-spectrum Antiviral Activity against Dengue and Zika Virus**

Merel Oeyen, M.S.[1], Dominique Schols, Ph.D.[1]

[1]Laboratory of Virology and Chemotherapy, Rega Institute, KU Leuven, Belgium, Leuven, Vlaams-Brabant, Belgium

9:25 AM **219.** **The Therapeutic Human Bispecific Neutralizing Antibody FIT-1 Treats Congenital Zika Disease**

Justin Julander, Ph.D.[1], Venkatraman Siddharthan, Ph.D.[1], Karin Stettler, Ph.D.[2], Elisabetta Cameroni, Ph.D.[2], John Morrey, Ph.D.[3], Davide Corti, Ph.D.[2]

[1]Institute for Antiviral Research, Utah State University, Logan, Utah, United States of America; [2]Huambs BioMed SA, a subsidiary of Vir Biotechnology, Bellinzona, Switzerland; [3]Institute for Antiviral Research, Logan, Utah, United States of America

9:30 AM **213.** **Towards the Development of Direct-Acting Antivirals for the Treatment of Human Parechoviruses Infections**

Kristina Lanko, M.S.[1], Yipeng Ma, M.S.[1], Leen Delang, Ph.D.[1], Carmen Mirabelli, Ph.D.[1], Johan Neyts, Ph.D.[1]

[1]KU Leuven, Rega Institute for Medical Research, Leuven, Belgium

9:40 AM **199. CMX521: A Nucleoside with Pan-Genotypic Activity against Norovirus**

Randall Lanier, Ph.D.[1], Dean Selleseth, B.S.[1], Abimbola Kolawole, Ph.D.[2], Myra Hosmillo, Ph.D.[3], Komal Nayak, M.S.[3], Andrew Bae, B.S.[1], Sarah Gurley, M.S.[1], Tim Tippin, Ph.D.[1], Heidi Colton, M.S.[1], John Dunn, Ph.D.[1], Mark Mullin, B.S.[1], Melissa Jones, Ph.D.[4], Stephanie Karst, Ph.D.[4], Brent Korba, Ph.D.[5], Matthias Zilbauer, M.D., Ph.D.[3], Ian Goodfellow, Ph.D.[3], Christiane Wobus, Ph.D.[2], Phiroze Sethna, Ph.D.[1]

[1]Chimerix, Durham, North Carolina, United States of America; [2]University of Michigan, Ann Arbor, Michigan, United States of America; [3]University of Cambridge, Cambridge, United Kingdom of Great Britain and Northern Ireland; [4]University of Florida, Gainesville, Florida, United States of America; [5]Georgetown University, Washington DC, District of Columbia, United States of America

9:53 AM **198. Letermovir Resistance Analysis in a Clinical Trial of Cytomegalovirus Prophylaxis for Haematopoietic Cell Transplant Recipients**

Cameron Douglas, Ph.D.[1], Diane Levitan, Ph.D.[2], Maureen Maguire, M.S.[2], Lei Chen, M.S.[2], Bo Wei, M.S.[2], Richard Barnard, Ph.D.[1], David Nickle, Ph.D.[3], Sunwen Chou, M.D.[4]

[1]Infectious Disease Research, Merck & Co., Inc., Kenilworth, New Jersey, United States of America; [2]Translational Molecular Biomarkers, Merck & Co., Inc., Kenilworth, New Jersey, United States of America; [3]Pharmacogenomics and Genetics, Merck & Co., Inc., Kenilworth, New Jersey, United States of America; [4]Department of Veterans Affairs Medical Center, Portland, Oregon, United States of America

Coffee Break

RIBEIRA I & II

10:30 AM – 11:00 AM

DNA Viruses and Emerging Viruses Session

Chairs: **Jennifer Moffat** and **Dana Wolf**

ARCHIVE HALL I

11:00 AM – 12:30 PM

11:00 AM **73. Broad Spectrum Virucidal Non-toxic Strategies**

Valeria Cagno, Ph.D.[1], Samuel Jones, Ph.D.[2], Ozgun Kocabiyik, M.S.[2], Matej Janecek, Ph.D.[2], Francesco Stellacci, Ph.D.[2], Caroline Tapparel, Ph.D.[3]

[1]University of Geneva and EPFL, Geneva, Geneva, Switzerland; [2]EPFL, Lausanne, Switzerland; [3]University of Geneva, Geneva, Switzerland

11:12 AM **172. Metabolic Activation of MBX-2168 Involves Enzymatic Removal of a Butyl-Ether Moiety by Adenosine Deaminase-Like Protein 1**

Anna Burns, B.S.[1], Hannah Sauer, B.S.[1], John Williams, Ph.D.[2], Marc Busch, Ph.D.[3], Terry Bowlin, Ph.D.[2], **Brian Gentry, Ph.D.**[1]

[1]Drake University College of Pharmacy and Health Sciences, Des Moines, Iowa, United States of America; [2]Microbiotix, Inc., Worcester, Massachusetts, United States of America; [3]Drake University College of Arts and Sciences, Department of Biology, Des Moines, Iowa, United States of America

11:24 AM **212. A Novel Class of Tryptophan Dendrimers Binds to the 5-fold Vertex of Enterovirus A71 Capsid and Blocks Virus Entry**

Liang Sun, M.S.[1], Hyunwook Lee, Ph.D.[2], Belen Martinez-Gualda, Ph.D.[3], Eva Rivero-Buceta, Ph.D.[3], Leen Delang, Ph.D.[4], Pieter Leyssen, Ph.D.[4], Ana San-Félix, Ph.D.[3], Susan Hafenstein, Ph.D.[2], Carmen Mirabelli, Ph.D.[4], Johan Neyts, Ph.D.[4]

[1]Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, KU Leuven, Leuven, Belgium; [2]Department of Biochemistry and Molecular Biology, Penn State College of Medicine; [3]Instituto de Química Médica (CSIC), Madrid, Spain; [4]Rega Institute for Medical Research, KU Leuven

11:36 AM **208. A Host-Targeting Antiviral Inhibitor, STF1019, Provides Protection against Enterovirus 71 Infection in a Mouse Model**

E. Bart Tarbet, Ph.D.[1], Brett Hurst, M.S.[1], W. Joseph Evans, M.S.[1], Christopher Peterson, B.S.[1], Mark Smith, Ph.D.[2], Khanh Nguyen, B.S.[2], Jeffrey Glenn, M.D., Ph.D.[2]
[1]Institute for Antiviral Research, Utah State University, Logan, Utah, United States of America; [2]Department of Medicine/Gastroenterology and Hepatology, Stanford University, Palo Alto, California, United States of America

11:48 AM **217. Identification of Small Chemical Compounds Blocking Tegumentation and Assembly of Herpes Simplex Virus**

Julio Cesar Villalvazo Guerrero, M.S.[1], Anna Buch, Ph.D.[1], Dorcas Otoo, M.S.[1], Anja Pohlmann, B.S.[1], Guillaume Beauclair, Ph.D.[1], Jessica Rückert, B.S.[1], Thomas Schulz, Ph.D.[1], Sodeik Beate, Ph.D.[1]
[1]Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover, Niedersachsen, Germany

12:00 PM **203. The Innovative Artemisinin Derivative Artemisone Is a Potent Inhibitor of Human Cytomegalovirus Replication**

Esther Oiknine-Djian, M.S.[1], Yiska Weisblum, Ph.D.[2], Amos Panet, Ph.D.[3], Ho Wong, M.D., Ph.D.[4], Richard Haynes, Ph.D.[4], **Dana Wolf, M.D**.[5]
[1]Hadassah University Hospital, IMRIC, Faculty of Medicine, Lautenberg Center for General and Tumor Immunology, Jerusalem, Israel; [2]IMRIC, The Hebrew University Faculty of Medicine, Jerusalem, Israel; [3]IMRIC, faculty of Medicine, Jerusalem, Israel; [4]Pharmacen, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa; [5]Hadassah University Hospital, The Lautenberg Center for General and Tumor Immunology, Jerusalem, Israel

Closing Remarks

Posters

In Numerical Order

1. Antiviral Investigation, High Performance Liquid Chromatography Analysis and Phytochemical Profiling of *Bryophyllum pinnatum* and *Viscum album*

Robert Obi, Ph.D.[1], Olumuyiwa Salu, Ph.D.[2], Abdul-Azeez Anjorin, Ph.D.[3], Bamidele Oke, Ph.D.[2], Juliet Shenge, Ph.D.[4], Ayorinde James, Ph.D.[5], Sunday Adesegun, Ph.D.[6], Sunday Omilabu, Ph.D.[7]

[1]Federal University of Technology, Owerri, Imo State, Owerri, Nigeria; [2]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos, Nigeria; [3]Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria; [4]Department of Virology, College of Medicine, University of Ibadan, Ibadan, Nigeria; [5]Department of Biochemistry, College of Medicine, University of Lagos, Lagos, Nigeria; [6]Department of Pharmacognosy, School of Pharmacy, University of Lagos, Lagos, Nigeria; [7]Department of Medical Microbiology and Parasitology, University of Lagos, Lagos, Nigeria

2. Susceptibility of Paramyxoviruses and Filoviruses to Inhibition by 2′-Monofluoro- and 2′-Difluoro-4′-Azidocytidine Analogs

Michael Lo, Ph.D.[1], Paul Jordan, Ph.D.[2], Sarah Stevens, M.S.[2], Yuen Tam, Ph.D.[2], Jerome Deval, Ph.D.[2], Stuart Nichol, Ph.D.[1], Christina Spiropoulou, Ph.D.[1]

[1]Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America; [2]Alios BioPharma, Inc., a Janssen Pharmaceutical Company of Johnson & Johnson, South San Francisco, California, United States of America

3. Several Mechanisms Contribute to Photodynamic Inhibition of HSV-1 Infection

Maja Cokarić Brdovčak, Ph.D.[1], Lara Djaković, M.S.[1], Ivana Bertović, M.S.[2], Martin Lončarić, Ph.D.[3], Antonija Jurak Begonja, Ph.D.[2], Nela Malatesti, Ph.D.[4], Igor Jurak, Ph.D.[1]

[1]Laboratory for Molecular Virology, Department of Biotechnology, University of Rijeka, Croatia, Rijeka, Croatia; [2]Laboratory for Haemathopoiesis, Department of Biotechnology, University of Rijeka, Croatia, Rijeka, Croatia; [3]Photonics and Quantum Optics Unit, Institute Ruder Bošković, Zagreb; [4]Laboratory for organic and solid state chemistry, Department of Biotechnology, University of Rijeka, Croatia, Rijeka, Croatia

5. Screening and Distribution of Different Genes Related to Cytoskeleton and Neuronal Transmitters in Hippocampus under Rabies Infection

Waqas Ahmad, Ph.D.[1], Zhang Maolin, Ph.D.[2], Ming Duan, Ph.D.[2], Iahtasham Khan, Ph.D.[3], Muhammad Awais, Ph.D.[3]

[1]Epidemiology and Public Health, College of Veterinary and Animal Sciences, Jhang, Punjab, Pakistan; [2]College of Veterinary Medicine, Institute of Zoonosis, Jilin University, Changchun, Jilin, China; [3]Section of Epidemiology and Public Health, College of Veterinary and Animal Sciences, Jhang, Punjab, Pakistan

6. Inactivation Effects of Calcium Hydrogen Carbonate Mesoscopic Crystals On Enveloped or Non-Enveloped Animal DNA and RNA Viruses.

Rikio Kirisawa, D.V.M., Ph.D.[1], Koichi Furusaki, Ph.D.[2], Rumiko Onishi, B.S.[2], Hoang Vu Dang, D.V.M., Ph.D.[3], Takashi Onodera, D.V.M., Ph.D.[4]

[1]Rakuno Gakuen University, Ebetsu, Hokkaido, Japan; [2]Mineral Activation Technical Research Center, Omuta, Fukuoka, Japan; [3]National Institute of Veterinary Research, Hanoi, Viet Nam; [4]The University of Tokyo, Tokyo, Japan

7. Identification of Compounds With Effect On Cell Proliferation, Apoptosis and Viral Tax Expression in HTLV-1-Infected Cell Line

Daiane Fernanda dos Santos, M.S.[1], Denise Regina de Pilger, M.S.[2], Charlotte Vandermeulen, M.S.[3], Ricardo Khouri, Ph.D.[4], Susimaire Mantoani, Ph.D.[5], Ivone Carvalho, Ph.D.[6], Jorge Casseb, Ph.D.[7], Jean-Claude Twizere, Ph.D.[8], Luc Willems, Ph.D.[9], Lucio H. Freitas-Junior, Ph.D.[10], Simone Kashima, Ph.D.[11]

[1]Faculty of Medicine of Ribeirão Preto, University of São Paulo; Blood Center of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil; [2]Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, São Paulo, São Paulo,

Brazil; [3]Protein Signaling and Interactions (GIGA), University of Liège, Liège, Belgium; [4]Gonçalo Moniz Research Center (CPqGM), Oswaldo Cruz Foundation (FIOCRUZ), Salvador, Bahia, Brazil; [5]School of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; [6]School of Pharmaceutical Sciences, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; [7]Institute of Tropical Medicine of São Paulo, University of São Paulo, São Paulo, São Paulo, Brazil; [8]Protein Signaling and Interactions (GIGA), University of Liège, Liège, Belgium; [9]Molecular and Cellular Epigenetics (GIGA), University of Liège, Liège, Belgium; [10]Federal University of São Paulo, São Paulo, São Paulo, São Paulo, Brazil; [11]Faculty of Medicine of Ribeirão Preto, University of São Paulo; Blood Center of Ribeirão Preto, Ribeirão Preto, São Paulo, Brazil

8. Comparison of Two Methods for Igm Anti-HAV Antibodies Measurement

Nafija Serdarevic, Ph.D.[1]
[1]Clinical center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

9. ACBD3, a Pan-Enterovirus Host Factor That Scaffolds Enterovirus 3A Proteins and the Host Factor PI4KB

Heyrhyoung Lyoo, M.S.[1], Hilde van der Schaar, Ph.D.[1], Cristina Dorobantu, Ph.D.[1], Jeroen Strating, Ph.D.[1], Frank van Kuppeveld, Ph.D.[1]
[1]Utrecht University, Utrecht, Utrecht, Netherlands

10. Eukariotic Ddx3 Helicase, a Potential Target for Virus Treatment

Marina Kukhanova, Ph.D.[1]
[1]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation

11. The Determination of Antimicrobial Sensitivity On Resistance of Bacteria

Nafija Serdarevic, Ph.D.[1]
[1]Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

12. Repurposing Heparin and Its Derivatives to Prevent ZIKA Virus-Induced Cell Death in Human Three-Dimensional (3D) Neurospheres (NS) Derived From Pluripotent Stem Cells

Isabel Pagani, M.S.[1], Elisa Vicenzi, Ph.D.[1]
[1]San Raffaele Scientific Institute, Milan, Italy

13. The Value Ferritin and Lactate in CSF in Patients With Meningitis

Nafija Serdarevic, Ph.D.[1]
[1]Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

14. Identification of Novel Interferon Gamma-Induced, Anti-Retrovirus Host Factors

Yoshinao Kubo, Ph.D.[1], Mai Izumida, M.D., Ph.D.[2], Yuria Umemura, B.S.[2], Hideki Hayashi, M.D., Ph.D.[3], Toshifumi Matsuyama, M.D., Ph.D.[4]
[1]Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Nagasaki University; [2]Department of Clinical Medicine, Institute of Tropical Medicine, Nagasaki University; [3]Medical University Research Administrator (MEDURA), Nagasaki University; [4]Department of Molecular Microbiology and Immunology, Nagasaki University

15. Chikungunya Virus Nsp3 Associates With the Lipid Kinase Sphingosine Kinase 2

St Patrick Reid, Ph.D.[1], Sarah Tritsch, M.S.[2], Dianne Wellems, M.S.[1]
[1]University of Nebraska Medical Center, Omaha, Nebraska, United States of America; [2]United States Army Research Institute of Infectious Diseases, Frederick, Maryland, United States of America

16. Heteroarylpyrimidine (HAP) and Novel Non-HAP Capsid Assembly Modifiers Show Differences in their Mode of Action *In Vitro*

Angelica Corcuera, M.S.[1], Katharina Stolle, B.S.[1], Stefan Hillmer, Ph.D.[2], Stefan Seitz, Ph.D.[3], Ralf Bartenschlager, Ph.D.[3], Alexander Birkmann, Ph.D.[1], Andreas Urban, Ph.D.[1]
[1]AiCuris Anti-Infective Cures GmbH, Wuppertal, Germany; [2]Electron Microscopy Core Facility, Heidelberg University, Heidelberg, Germany; [3]Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany

17. IMB-Z Inhibits Enterovirus 71 Replication Through Regulating the Host Apolipoprotein B Messenger RNA-Editing Enzyme Catalytic Polypeptide-Like 3G

Huiqiang Wang, M.S.[1], Yanping Li, Ph.D.[1], Ke Li, Ph.D.[1], Shuo Wu, Ph.D.[1], Haiyan Yan, Ph.D.[1], Danqing Song, Ph.D.[1], Yuhuan Li, Ph.D.[1]
[1]Institute of Medicinal Biotechnology, Beijing, China

18. Targeting RNA Methyltransferase METTL3 Reverses Drug Resistance in Influenza A Virus

Ali Ozes, Ph.D.[1], Andreas Jekle, Ph.D.[1], Leonid Beigelman, Ph.D.[1]
[1]Alios BioPharma, Inc., part of the Janssen Pharmaceutical Companies., South San Francisco, California, United States of America

19. Development and Validation of a High-Throughput Screen to Identify Macrocyclic Inhibitors Targeting the Zika Virus E Protein

Jared Pitts, Ph.D.[1], Wenlong Lian, Ph.D.[1], Priscilla Yang, Ph.D.[1]
[1]Department of Microbiology and Immunobiology, Harvard Medical School, Boston, Massachusetts, United States of America

20. Antiviral and Anti-Inflammatory Activity of Budesonide against Human Rhinovirus Infection Mediated Via Autophagy Activation

Jae-Hyoung Song, Ph.D.[1], Seong-Ryeol Kim, M.S.[1], Jae-Hee Ahn, B.S.[1], Sun-Young Chang, Ph.D.[2], Hyun-Jeong Ko, Ph.D.[1]
[1]Laboratory of Microbiology and Immunology, College of Pharmacy, Kangwon National University, Korea (Republic of); [2]College of Pharmacy, Ajou University

21. A High-Throughput Screening of FDA-Approved Drugs and Small Molecular Library for Broad-Spectrum Inhibitors of Coronaviruses Infection

Liang Shen, Ph.D.[1], **Wenjie Tan, Ph.D.**[2]
[1]Key Laboratory of Medical Virology, Ministry of Health, National Institute for Viral Disease Control and Prevention, Beijing, China; [2]National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China

22. Identification of Novel Drugs against Yellow Fever Using High Content Screening

Denise R Pilger, B.S.[1], Carolina Moraes, Ph.D.[1], Sabrina R Queiroz, Ph.D.[2], Paolo M Zanotto, Ph.D.[1], Laura H Gil, Ph.D.[3], Lucio Freitas-Junior, Ph.D.[1]
[1]Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil; [2]Sao Paulo Universtity, Pirassununga, Brazil; [3]CPqAM-Fiocruz, Universidade Federal de Pernambuco, Recife, Pernambuco, Brazil

23. Development and Characterization of Surface Modified Chitosan Nanoparticles for Selective Targeting of Lamivudine to Hepatocyte

Saurabh Bhargava, M.D., MPH[1], Vishal Bhargava, Ph.D.[2]
[1]United Institute of Pharmacy, India; [2]GTB Hospital, India

24. High Content Screening/Imaging of Differentiated Human Primary Neural Cells Identifies Drug Candidates That Inhibit TBEV Infection.

Nathalie Aulner, Ph.D.[1], Mazigh Fares, M.S.[2], Marielle Cochet, M.S.[2], Anne Danckaert, Ph.D.[1], Muriel Coulpier, Ph.D.[2]
[1]Institut Pasteur, Paris, France; [2]UMR 1161-INRA-ANSES-EnvA, Maisons-Alfort, France

25. Development of First-In-Class Encephalitic Alphavirus Inhibitors With In Vivo Efficacy

Jennifer Golden, Ph.D.[1], Donghoon Chung, Ph.D.[2], Jon Gabbard, Ph.D.[2], Colleen Jonsson, Ph.D.[3]
[1]University of Wisconsin-Madison, Madison, Wisconsin, United States of America; [2]University of Lousiville, Louisville, Kentucky, United States of America; [3]University of Tennessee Health Science Center, Memphis, Tennessee, United States of America

26. C2 and C7 Arylated Tryptophan Trimers and Tetramers As Dual HIV and Enterovirus 71 Entry Inhibitors

Belén Martínez-Gualda, Ph.D.[1], Olaia Martí, M.S.[1], Sofía de la Puente, M.S.[1], Liang Sun, M.S.[2], Carmen Mirabelli, Ph.D.[2], Johan Neyts, Ph.D.[2], Dominique Schols, Ph.D.[2], María-José Camarasa, Ph.D.[1], **Ana San-Félix, Ph.D.**[1]

[1]medicinal Chemistry Institute-Spanish Research Council, Madrid, Madrid, Spain; [2]rega Institute For Medical Research-University Of Leuven, Leuven, Belgium

27. 2,4,6-Trisubstituted Pyrimidines as Potent HIV Non-nucleoside Reverse Transcriptase Inhibitors

Petr Simon, Ph.D.[1], Lucie Cechova, M.S.[1], Ondrej Baszczynski, Ph.D.[1], Zlatko Janeba, Ph.D.[1], David Saman, Ph.D.[1], George Stepan, Ph.D.[2], Eric Hu, Ph.D.[2], Eric Lansdon, Ph.D.[2], Petr Jansa, Ph.D.[2]

[1]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences; [2]Gilead Sciences Inc.

28. Protective Efficacy of a ZIKV VLP Vaccine in a Lethal Mouse Model

Justin Julander, Ph.D.[1], Lo Vang, Ph.D.[2], Carla Uranga, Ph.D.[2], Ben Guenther, B.S.[2], Jayavani Aruri, M.S.[2], Danielle Thompson, M.S.[2], Angela Clyde, B.S.[1], Jeff Alexander, Ph.D.[2]

[1]Institute for Antiviral Research, Utah State University, Logan, Utah, United States of America; [2]PaxVax, San Diego, California, United States of America

29. Progress and Resources of NIAID's Antiviral Development against Biodefense and Emerging Viral Diseases

Helen Schiltz, Ph.D.[1]

[1]Office of Biodefense, Research Resources, and Translational Research (OBRRTR), DMID, NIAID, Bethesda, Maryland, United States of America

30. Targeting HIV-Infected Brain to Improve Stroke Outcome

Luc Bertrand, Ph.D.[1], Fannie Méroth, B.S.[1], Michal Toborek, M.D., Ph.D.[1]

[1]University of Miami - Miller School of Medicine, Miami, Florida, United States of America

31. Chiral Substituted Pyrimidines As Potent HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors

Lucie Čechová, M.S.[1], Ondřej Baszczynski, Ph.D.[1], David Šaman, Ph.D.[1], George Stepan, Ph.D.[2], Eric Hu, Ph.D.[2], Eric Landson, Ph.D.[2], Petr Jansa, Ph.D.[2], Zlatko Janeba, Ph.D.[1], Petr Šimon, Ph.D.[1]

[1]IOCB CAS, Prague, Czechia; [2]Gilead Sciences Inc

33. The Hepatitis C Virus RNA-Dependent RNA Polymerase Shuttles Incoming Nucleotides to the Active Site Through Successive Local Magnesium-Dependent Rearrangements

Kaouther Ben Ouirane, Ph.D.[1]

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34. *In Vitro* Identification of a Src-Family Kinase Inhibitor As a New Therapeutic Option for Middle East Respiratory Syndrome Coronavirus Infection

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35. 2-Thiouracil Derivatives Downregulate Human Adenovirus Replication

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36. Computer-Aided Discovery and Characterization of Novel Ebola Virus Inhibitors

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37. The Novel Nucleoside Analogue LJ-4269 Inhibits Coronavirus Replication.

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38. Structure-Activity Relationship Study of Itraconazole, a Broad-Range Inhibitor of Enterovirus Replication That Targets Oxysterol-Binding Protein

Lisa Bauer, M.S.[1], Salvatore Ferla, Ph.D.[2], Sarah Head, Ph.D.[3], Shridhar Bhat, Ph.D.[3], Kalyan Pasunooti, Ph.D.[3], Wei Shi, Ph.D.[3], Lucian Albulescu, M.S.[1], Jun Liu, Ph.D.[3], Andrea Brancale, Ph.D.[2], Frank van Kuppeveld, Ph.D.[1], Jeroen Strating, Ph.D.[1]
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39. 39. Understanding Flavivirus Pathogenesis: Hijacking of Human Proteins by Non-Coding Viral RNA

Sander Jansen, M.S.[1], Johan Neyts, Ph.D.[1], Kai Dallmeier, Ph.D.[1]
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40. Viral RNA Modification

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41. The *In Vitro* Characterisation of RV521, a small molecule Respiratory Syncytial Virus Fusion Inhibitor in Clinical Development

Claire Scott, Ph.D.[1], Rebecca Dowey, M.S.[1], Dan Brookes, Ph.D.[1], Molly Steadman, M.S.[1], Elaine Thomas, Ph.D.[1], Neil Mathews, Ph.D.[1], Alexandre Bedernjak, Ph.D.[1], Matthew Barrett, Ph.D.[1], Stuart Cockerill, Ph.D.[1], Ken Powell, Ph.D.[1], Eddy Littler, Ph.D.[1]
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42. The Natural Compound Silvestrol, a Selective Inhibitor of the RNA Helicase Eif4a, Has Potent Broad-Spectrum Antiviral Activity

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43. Anti-Influenza Efficacy of Combined Use Aminocaproic Acid With the Benzodiazols' Derivatives *In Vitro*

Tetyana Grydina, Ph.D.[1], Alla Fedchuk, Ph.D.[2], Larisa Shitikova, M.S.[3], Viktor Lozitsky, Ph.D.[4], Viktor Kuz'min, Ph.D.[5], Anatoly Artemenko, Ph.D.[6], Stepan Basok, Ph.D.[6], Olexandr Gruzevskiy, Ph.D.[7]
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44. Oligomerization Dynamics and Thermodynamics of Wt and Triple-Mutant Ebola VP35 Coiled-Coil Region From Self-Assembly Simulations

Francesco Di Palma, Ph.D.[1], Venkata Krishnan Ramaswamy, Ph.D.[1], Gianluca Daino, Ph.D.[2], Aldo Frau, Ph.D.[2], Angela Corona, Ph.D.[2], Luca Zinzula, Ph.D.[3], Attilio Vargiu, Ph.D.[1], Enzo Tramontano, Ph.D.[2], Paolo Ruggerone, Ph.D.[1]

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45. Neplanocin A Derivatives As Selective Inhibitors of HBV Transcription

Masaaki Toyama, Ph.D.[1], Takayuki Hamasaki, Ph.D.[1], Mika Okamoto, M.D., Ph.D.[1], Masanori Ikeda, M.D., Ph.D.[2], Koichi Watashi, Ph.D.[3], Takaji Wakita, M.D., Ph.D.[3], Atsuya Yamashita, Ph.D.[4], Kohji Moriishi, D.V.M., Ph.D.[4], Ashoke Sharon, Ph.D.[5], Masanori Baba, M.D., Ph.D.[1]

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47. CRISPR/Cas9: A Molecular Tool to Selectively Target Merkel Cell Carcinoma.

Dimitrios Topalis, Ph.D.[1], Graciela Andrei, Ph.D.[1], Robert Snoeck, M.D., Ph.D.[1]

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48. Posaconazole Inhibits Dengue Virus Replication by Targeting Oxysterol-Binding Protein

Febrina Meutiawati, M.S.[1], Bodine Bezemer, M.S.[1], Jeroen R.P.M Strating, Ph.D.[2], Eva Žusinaite, M.D.[3], Frank J.M. van Kuppeveld, M.D., Ph.D.[2], Koen van Cleef, Ph.D.[4], Ronald van Rij, Ph.D.[1]

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49. The Host-Targeted Iminosugar UV-4B Inhibits Influenza Virus without Selecting for Resistance

Lisa Evans DeWald, Ph.D.[1], Urban Ramstedt, Ph.D.[2], Dale Barnard, Ph.D.[3], Michelle Mendenhall, Ph.D.[3], Eric Stavale, M.S.[4], Suman Das, Ph.D.[2], Kelly Warfield, Ph.D.[1]

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50. Identification of Obatoclax as Broad-Spectrum Antiviral Preventing Entry through Acidic Endosomes

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51. HBV RNA Is an Early Predictor of Sustained HBeag Seroconversion in HBeAg-Positive Patients Treated with Pegylated Interferon Alpha

Min Zhang, M.D., Ph.D.[1], **Guangdi Li, Ph.D.**[2], Jia Shang, M.D., Ph.D.[3], Chen Pan, M.D., Ph.D.[4], Minxiang Zhang, M.D., Ph.D.[5], Chibiao Yin, M.D., Ph.D.[6], Qing Xie, M.D., Ph.D.[7], Yanzhong Peng, M.D., Ph.D.[8], Qing Mao, M.D., Ph.D.[9], Xinqiang Xiao, B.S.[10], Yongfang Jiang, M.D., Ph.D.[11], Kaizhong Luo, M.D., Ph.D.[12], Hai Ding, Ph.D.[13], Vidaurre Diego, Ph.D.[14], Mahmoud Reza Pourkarim, Ph.D.[15], Erik De Clercq, M.D., Ph.D.[16], Guiqiang Wang, M.D., Ph.D.[17], Guozhong Gong, M.D., Ph.D.[18]

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52. A Rhinovirus Early Stage Inhibitor with a Novel Mechanism of Action

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53. Aniline-Based Inhibitors of Influenza H1N1 Virus Acting On Hemagglutinin-Mediated Fusion

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54. Inhibition of Influenza and Human Corona Viruses by 1,4,4-Trisubstituted Piperidines

Sonsoles Velázquez, Ph.D.[1], De Castro Sonia, Ph.D.[1], Cumella José, Ph.D.[1], Laporte Manon, Ph.D.[2], Evelien Vanderlinden, Ph.D.[2], Annelies Stevaert, Ph.D.[2], Lieve Naesens, Ph.D.[2], María-José Camarasa, Ph.D.[1]
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55. Discovery of Broad-Spectrum Influenza Antivirals by Targeting Pro-Viral Host Factors

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56. Verdinexor, a Novel Inhibitor of Exportin-1, Shows *In Vitro* Efficacy against Opportunistic Viral Infections in the Immunocompromised Patient

Douglas Widman, Ph.D.[1], Savanna Gornisiewicz, B.S.[1], Sharon Shacham, Ph.D.[2], Sharon Tamir, M.S.[1]
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57. A New Locus of Letermovir Resistance in the Human Cytomegalovirus UL56 Gene Revealed by In Vitro Exposure to Letermovir and Ganciclovir

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58. Deubiquitinating and Inhibition of Porcine Epidemic Diarrhea Virus Papain-like Protease 2

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59. 3′-Halo-5′-norcarbocyclic nucleoside phosphonates as potent anti-HIV agents

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60. Antiviral Activity of a Series of Indole Alkaloids against Emerging Flaviviruses

Dominique Schols, Ph.D.[1], **Antonios Fikatas, M.S.**[2]
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61. Identification of a Druggable VP1-VP3 Interpromoter Binding Pocket in the Capsid of Enteroviruses.

Rana Abdelnabi, Ph.D.[1], James Geraets, Ph.D.[2], Yipeng Ma, M.S.[1], Carmen Mirabelli, Ph.D.[1], Ajay Kumar Timiri, M.S.[3], Justin Flatt, Ph.D.[2], Aušra Domanska, M.S.[2], Leen Delang, Ph.D.[1], Dirk Jochmans, Ph.D.[1], Venkatesan Jayaprakash, Ph.D.[3], Barij Nayan Sinha, Ph.D.[2], Pieter Leyssen, Ph.D.[1], Sarah Butcher, Ph.D.[2], Johan Neyts, Ph.D.[1]
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62. Novel PI4KB Inhibitor- Potent and Broadspectrum Antivirals against Enterovirus

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63. Verdinexor (KPT-335) Demonstrates Antiviral Activity against Multiple Emerging Influenza Strains

Savanna Gornisiewicz, B.S.[1], Rebecca Dewar, Ph.D.[2], Douglas Widman, Ph.D.[1], Sharon Shacham, Ph.D.[1], Paul Digard, Ph.D.[3], Sharon Tamir, M.S.[1]
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64. The Viral Polymerase Inhibitor 7DMA reduces Zika Virus Replication in Reproductive Organs of Male Mice

Sofie Jacobs, M.S.[1], Suzanne Kaptein, Ph.D.[1], Leen Delang, Ph.D.[1], Johan Neyts, Ph.D.[1]
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65. An Enterovirus Infection Model of the Murine Upper Respiratory Tract to Assess the Activity of Antivirals

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66. Genetic Variants Analysis of BK Polyomavirus Genome and of the Cellular *CMPK1* Gene from Kidney Transplant Recipients

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67. Analysis of Cidofovir (CDV) as an Anti-Tumor Drug and Evaluation of the Mechanisms Underlying CDV Resistance by Comparing HPV16+ Cervical Carcinoma (Siha), Siha CDV-Resistant ($Siha_{cdv}$) and Normal (Primary Human Keratinocytes - Phks) Cells

Tatiane de Araujo Nogueira, Ph.D.[1], Barbara Mertens, Ph.D.[1], Ruzena Stranska, Ph.D.[1], Dimitri Topalis, Ph.D.[1], Graciela Andrei, Ph.D.[1], Robert Snoeck, M.D., Ph.D.[1]
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68. **Nitazoxanide Inhibits Human Norovirus Replication and Synergizes with Ribavirin by Activation of Cellular Antiviral Response**

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69. **A Systematic Analysis of the Potential and Limitations of Epigenetic Inhibitors As Antiviral Drugs**

Bizhan Romani, Ph.D.[1], **Luis Schang, D.V.M., Ph.D.**[1]
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70. **Bipolymer Based Novel Nanoparticles in Microsphere System As Vaccine Adjuvant**

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71. **Antibody coated Liposomes for Transmucosal vaccination**

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[1]Dr. H S Gour University, Sagar, India; [2]United Institute of Pharmacy, Allahabad, India

72. **Galidesivir (BCX4430) Limits Rift Valley Fever Virus Infection and Disease Modeled in Syrian Golden Hamsters**

Jonna Westover, Ph.D.[1], Amanda Mathis, Ph.D.[2], Ray Taylor, M.S.[2], Luci Wandersee, B.S.[1], Kevin Bailey, B.S.[1], Eric Sefing, M.S.[1], Brady Hickerson, B.S.[1], Kie-Hoon Jung, Ph.D.[1], William Sheridan, M.D.[2], **Brian Gowen, Ph.D.**[1]
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73. **Broad Spectrum Virucidal Non Toxic Strategies**

Valeria Cagno, Ph.D.[1], Samuel Jones, Ph.D.[2], Ozgun Kocabiyik, M.S.[2], Matej Janecek, Ph.D.[2], Francesco Stellacci, Ph.D.[2], Caroline Tapparel, Ph.D.[3]
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74. **Impact of Hiv-1 Subtype and Korean Red Ginseng On Aids Progression: Comparison of Subtype B and Subtype D**

Young-Keol Cho, M.D., Ph.D.[1], Jung-Eun Kim, M.S.[1], Brian Foley, Ph.D.[2]
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75. **Pemetrexed Inhibits Human Herpesviruses Through Reactivation of P53**

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76. **Type I Interferon Induced by a Recombinant Adenovirus Expressing Three Shrnas Enhanced Antiviral Effects against Foot-And-Mouth Disease Virus**

Su-mi Kim, Ph.D.[1], Ji-hyeon Hwang, M.S.[1], Jong-hyeon Park, Ph.D.[1], Min-ja Lee, Ph.D.[1], Byounghan Kim, Ph.D.[1]
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77. **Pomegranate Peel Extracts Possess a Promising Anti-influenza Virus Activity**

Malak Alame, M.S.[1], Amani Ezzeddine, M.S.[2], Fatima Saleh, Ph.D.[3], **Hassan Zaraket, Ph.D.**[4]
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78. Molecular Mechanism of Highly Potent NS5A Inhibitors

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79. Use of Recombinant Herpes Simplex Virus Strains to Characterize Novel UL23 Thymidine Kinase Mutations Toward Resistance to Acyclovir

David Boutolleau, M.D., Ph.D.[1], Thibaud Goupil-Gouyette, M.S.[1], Rachel Bellone, M.S.[1], Julien Marlet, M.D.[1], Sonia Burrel, M.D., Ph.D.[1]
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80. In Vitro Antiviral Activity of Stilbenes Isolated from *Macaranga barteri* against Enteroviruses

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81. Antiviral Potential of Fluorinated Analogs of Thymidine

Liubov Biliavska, Ph.D.[1], Yulia Pankivska, M.S.[1], Olga Povnitsa, Ph.D.[1], Svitlana Zagorodnya, Ph.D.[1], Yuriy Shermolovich, M.D.[2]
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82. Induction of Apoptosis in the EBV-Associated Cell by Fluorinated Derivatives

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83. Initial Studies on T-1105's Redox Properties and Its Structural Similarity to Nicotinamide

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84. Acyclovir-Resistant Herpetic Keratitis (HK) in an Immunocompetent Patient

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85. Human SAMHD1 Restricts the Xenotransplantation Relevant Porcine Endogenous Retrovirus (PERV)

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86. Biological Evaluation of Novel Small-Molecule Antiviral Agents Versus Chikungunya Virus

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87. Design of Effective Multivalent Influenza Virus Inhibitors Preventing Binding to Host Cells

Malte Hilsch, M.S.[1], Daniel Lauster, Ph.D.[1], Victor Bandlow, M.S.[1], Sumati Bhatia, Ph.D.[2], Oliver Seitz, Ph.D.[1], Kai Ludwig, Ph.D.[2], Christoph Böttcher, Ph.D.[2], Rainer Haag, Ph.D.[2], Andreas Herrmann, Ph.D.[1]
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88. Identification of Novel Norovirus Antiviral CMX521 Using High-Throughput Screening

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89. Synthesis and Evaluation of Tenofovir Diester Prodrugs as Potential Long-acting Agents for Treatment and Prevention of HIV-1 Infection

James Beadle, Ph.D.[1], Nadejda Valiaeva, Ph.D.[2], Xing-Quan Zhang, Ph.D.[1], Kathy Aldern, B.S.[1], Robert Schooley, M.D.[1], Karl Hostetler, M.D.[1]
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90. First Steps Towards Developing Novel Inhibitors of Enterovirus Polymerases

Clara van Hoey, M.S.[1], Laurence Jung, Ph.D.[2], Christophe Morice, Ph.D.[2], Jean-Marie Contreras, Ph.D.[2], Thomas Seidel, Ph.D.[1], Lisa Bauer, M.S.[3], Frank van Kuppeveld, Ph.D.[3], Thierry Langer, Ph.D.[1]
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91. Identification of Natural Product-Based Inhibitors Targeting Enterovirus 71 VP1-Receptor Interaction

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92. Evaluation of Severe Disease Mouse Model Caused by Highly Pathogenic Avian Influenza Infection

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93. Characterization of Murine Gammaherpesvirus-68 (MHV-68) Resistance and Fitness

Erika Trompet, M.S.[1], Sarah Gillemot, M.S.[1], Graciela Andrei, Ph.D.[1], Robert Snoeck, M.D., Ph.D.[1]
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94. Targeting Cyclophilin A to Block Viral Innate Immune Evasion and Engage Antiviral Immune Responses

Che Colpitts, Ph.D.[1], Bethany Schneiderman, B.S.[1], Justin Warne, Ph.D.[1], David Selwood, Ph.D.[2], Greg Towers, Ph.D.[1]
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95. High-Throughput Screening and Development of Antiviral Compound against Middle East Respiratory Syndrome–Coronavirus (MERS-Cov)

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96. Preliminary *In Vitro* Antiretroviral Activity of a *Nigella Sativa* Seed Formulation (A-Zam) against HIV-1

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97. A High Content Imaging-Based Neutralization (HINT) Assay to Assess Susceptibility of Influenza Viruses to Antivirals Targeting Virus Entry

Vasiliy Mishin, Ph.D.[1], Patty Jorquera, Ph.D.[1], John Barnes, Ph.D.[1], Ha Nguyen, Ph.D.[1], David Wentworth, Ph.D.[1], Larisa Gubareva, Ph.D.[1]
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98. Laboratory and Clinical Strains of Dengue Virus Differentially Affect Endothelial Cells

Peter Vervaeke, Ph.D.[1], Sam Noppen, M.S.[1], Eef Meyen, B.S.[1], Sandra Claes, B.S.[1], Kevin Ariën, Ph.D.[2], Sandra Liekens, Ph.D.[1]
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99. Divide Et Impera, Small Molecule Disruptors of Ul44 Dna Polymerase Processivity Factor: A New Class of Cytomegalovirus Inhibitors

Martina Timmoneri, M.S.[1], Veronica Di Antonio, Ph.D.[1], Federico Falchi, Ph.D.[2], Beatrice Mercorelli, Ph.D.[1], Arianna Loregian, Ph.D.[1], Giorgio Palù, M.D., Ph.D.[1], **Gualtiero Alvisi, Ph.D.**[1]
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100. Development and Characterization of a Recombinant Lassa Virus Expressing Enhanced Green Fluorescent Protein as a Tool for High-throughput Drug Screen and Neutralizing Antibody Assays

Yingyun Cai, Ph.D.[1], Masaharu Iwasaki, Ph.D.[2], Shuiqing Yu, B.S.[1], Elena Postnikova, Ph.D.[1], Beatrice Cubitt, M.S.[2], Lisa DeWald, Ph.D.[1], Sheli Radoshitzky, Ph.D.[3], Laura Bollinger, M.S.[1], Peter Jahrling, Ph.D.[1], Juan de la Torre, Ph.D.[2], Jens Kuhn, Ph.D.[1]
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101. Kinetic, Thermodynamic and Structural Analysis of the H275Y, I223V, and S247N Neuraminidase Resistant Mutants of the 2009 Pandemic Influenza Virus

Jana Pokorná, Ph.D.[1], Petr Pachl, Ph.D.[1], Pavlína Řezáčová, Ph.D.[1], Elena Karlukova, B.S.[1], Jakub Hejdánek, B.S.[1], Aleš Machara, Ph.D.[1], Jan Konvalinka, Ph.D.[1], Milan Kožíšek, Ph.D.[1]
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102. Combination of Enterovirus Inhibitors Delay or Prevent the Development of Enterovirus-A71 Resistant Variants

Kristina Lanko, M.S.[1], Chenyan Shi, Ph.D.[1], Shivaprasad Patil, Ph.D.[1], Leen Delang, Ph.D.[1], Jelle Matthijnssens, Ph.D.[1], Carmen Mirabelli, Ph.D.[1], Johan Neyts, Ph.D.[1]
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103. A New System for a Silent Virus: Developing a Skin Tissue Model for Human Cytomegalovirus

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104. Inhibition of Cytosolic Phospholipase A2alpha Impairs Coronavirus Replication by Interfering with Virus-Induced Replicative Organelle Formation

Christin Müller, M.S.[1], Martin Hardt, Ph.D.[1], Dominik Schwudke, Ph.D.[2], Benjamin Neuman, Ph.D.[3], Stephan Pleschka, Ph.D.[1], John Ziebuhr, Ph.D.[1]
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105. The Search for Novel Zika Virus Inhibitors and the Establishment of Relevant *In Vivo* Mouse Models to Evaluate their Efficacy

Haridian Montanez Brull, M.S.[1], Sofie Jacobs, M.S.[1], Leen Delang, Ph.D.[1], Suzanne Kaptein, Ph.D.[1], Johan Neyts, Ph.D.[1]
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106. Nanoviricides Prevent Varicella Zoster Virus Infection in Human Skin

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107. Screening Campaign for the Identification of Novel Inhibitors of the Porcine Reproductive and Respiratory Syndrome Virus

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108. Synthesis and Antiviral Eevaluation of Fluorescent T-1105 and T-1106 Analogues

Matthias Winkler, M.S.[1], Evelien Vanderlinden, Ph.D.[2], Lieve Naesens, Ph.D.[2], Chris Meier, Ph.D.[1]
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109. Oral Combination Vaccine against Anthrax & Hepatitis B: Development and Characterization

Sourabh Jain, M.D., MPH[1], Nikhil Kapoor, M.S.[2], Varun Bhargava, B.S.[3], Saurabh Bhargava, M.D., MPH[4]
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110. Some Medicinal Plants from Nigerian Ethnomedicine Exhibited Antiviral Activity against Enteroviruses

Toluwanimi Akinleye, B.S.[1], Omonike Ogbole, Ph.D.[1], Peter Segun, M.S.[1], Adekunle Adeniji, Ph.D.[2]
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111. Synthesis and Anti-HIV Activities of CD4 Down-modulating, Pyridine-fused CADA Compounds

Thomas Bell, Ph.D.[1], Liezel Lumangtad, Ph.D.[1], Sunil Hamal, Ph.D.[1], Dominique Schols, Ph.D.[2], Kurt Vermeire, Ph.D.[2]
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112. Prevalence of Hepatitis Infections in Type 1, Type 2 Diabetes and Latent Autoimmune Diabetes of Adults (LADA): Implications for Antiviral and Diabetes Treatments

Yujin Ding, M.S.[1], **Guangdi Li, Ph.D.**[1], Xiang Yan, M.S.[1], Xiaoli Zhang, B.S.[1], Xinqiang Xiao, B.S.[2], Bingwen Liu, M.S.[1], Xiaohan Tang, M.S.[1], Ying Xia, M.S.[1], Xing Gong, B.S.[2], Guozhong Gong, Ph.D.[2], Erik De Clercq, Ph.D.[3], Zhiguang Zhou, Ph.D.[1]
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113. Antiviral Properties of Titanium Dioxide Nanoparticles against Human Adenovirus Serotype 5

Yuliia Pankivska, Ph.D.[1], Maksym Zahornyi, Ph.D.[2], Liubov Biliavska, Ph.D.[1], Olga Povnitsa, Ph.D.[1], Andrey Ragulya, Ph.D.[2], Svitlana Zagorodnya, Ph.D.[1]
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114. Anti-HIV Activity of Flavonoid Glycosides in Extracts from Deschampsia Caespitosa L. and Calamagrostis Epigeios L. (*In Vitro* and *In Silico* Study)

Tatyana Trokhymchuk, M.S.[1], Svitlana Rybalko, M.D.[1], Daria Starosyla, Ph.D.[1], Olexander Vasylchenko, Ph.D.[2], Viktor Atamaniuk, M.D.[2], Svitlana Zagorodnya, Ph.D.[3], Michael Zavelevich, Ph.D.[4]
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115. Combination of Oseltamivir and a Novel Kinase Inhibitor has Synergistic Antiviral Activity against Influenza Viruses

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116. Effect of Double Antiviral Combinations Applied by Consecutive Alternating Administration against Coxsackievirus B1 Infection in Mice

Adelina Stoyanova, M.S.[1], Ivanka Nikolova, Ph.D.[1], Petya Stoyanova, D.V.M.[1], Stefan Philipov, Ph.D.[2], Gerhard Pürstinger, Ph.D.[3], Angel Galabov, M.D., Ph.D.[1]
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117. Biophysical Analysis of Amphipathic α-Helical (AH) Peptides

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118. Evaluation of the Antiviral Activity of Anisomycin against Dengue and Zika Viruses: *In Vitro* and *In Vivo* Studies.

Verónica Quintana, M.S.[1], Barbara Selisko, Ph.D.[2], Jesús Brunetti, M.S.[1], Cecilia Eydoux, Ph.D.[3], Jean Guillemot, Ph.D.[3], Bruno Canard, Ph.D.[3], Justin Julander, Ph.D.[4], Viviana Castilla, Ph.D.[1]
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119. N-acylhydrazones as RNase H Selective Inhibitors Active against Replication of HIV-1 NNRTIs Resistant Variants

Angela Corona, Ph.D.[1], Dominga Rogolino, Ph.D.[2], Ester Ballana, Ph.D.[3], Mauro Carcelli, Ph.D.[2], Nicole Grandi, Ph.D.[1], José Esté, Ph.D.[3], Enzo Tramontano, Ph.D.[1]
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120. Mapping the Antiviral Chemical Space

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121. Activity of Double Combinations of New Diaryl Ethers against Coxsackievirus B1

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122. *In Vitro* Antiviral Activity of λ Carrageenan against DENV Infection of Human Myeloid Cells in Absence or Presence of Antibodies

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123. Consecutive Alternating Treatment Strategy against Coxsackievirus B3 Infections

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124. esign, Synthesis and Evaluation of Novel Potential Antivirals Targeting the Norovirus Polymerase

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125. Development of a Highly Efficient Bioreactor for Production of Chicken Egg Yolk Antibodies (Igy) as a Prophylactic and Therapeutic Agent for Human Norovirus

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127. Family Outbreak of ECHO-3 Enterovirus Infection

Alexandra Sovkich, M.S.[1], Tamara Amrosieva, M.D., Ph.D.[2], Natallia Matsiyeuskaya, M.D., Ph.D.[1], Yullia Shilova, B.S.[2]
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128. The Ebolavirus VP35 Oligomerization Domain: Crystal Structures and Biophysical Characterization of a New Potential Antiviral Target

Luca Zinzula, Ph.D.[1], István Nagy, Ph.D.[1], Massimiliano Orsini, Ph.D.[2], Elisabeth Weyher-Stingl, Ph.D.[3], Andreas Bracher, Ph.D.[4], Wolfgang Baumeister, Ph.D.[1]
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129. Dual Effect of the Multi-Kinase Inhibitor Midostaurin on Acute and Latent HIV-1 Infection

Edurne Garcia-Vidal, M.S.[1], Marc Castellví, M.S.[1], Maria Pujantell, M.S.[1], Roger Badia, Ph.D.[1], Bonaventura Clotet, M.D., Ph.D.[1], Eva Riveira-Muñoz, Ph.D.[1], Ester Ballana, Ph.D.[1], José Esté, Ph.D.[1]
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130. Development of a "Hepatitis B Virus (HBV) Persistence" Mouse Model Amenable for Antiviral and Vaccine Evaluation

Junzhong Peng, M.S.[1], Kevin Walters, Ph.D.[1], Melanie Hussong, Ph.D.[2], Samuel Rulli, Ph.D.[2], Vinayaka Kotraiah, Ph.D.[3], Timothy Phares, Ph.D.[3], Gabriel Gutierrez, Ph.D.[3]
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131. Development and Characterization of an Aerodynamic System for Pulmonary Delivery of Influenza Vaccine

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132. Exploring Strategies to Improve Therapeutic Efficacy of Antiviral Drugs Targeting the N-linked Glycan Processing Pathway for Treatment of Viral Hemorrhagic Fevers

Yanming Du, Ph.D.[1], Julia Ma, B.S.[1], Xuexiang Zhang, M.S.[1], Travis Warren, Ph.D.[2], Veronica Soloveva, Ph.D.[2], Fang Guo, M.D., Ph.D.[1], Qing Su, Ph.D.[1], Nicky Hwang, B.S.[1], Shuo Wu, Ph.D.[1], Sina Bavari, Ph.D.[2], Ju-Tao Guo, M.D.[1], Timothy Block, Ph.D.[1], Jinhong Chang, M.D., Ph.D.[1]
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133. *Cuscuta reflexa* (Giant dodder): Potential Inhibitor of HCV NS3 Serine Protease

Sobia Noreen, Ph.D.[1], Shazia Noureen, M.S.[1], Shazia Ghumman, Ph.D.[2], Fozia Batool, Ph.D.[1], Bushra Ijaz, Ph.D.[3], Qamar Zaman, M.S.[4]
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134. Successful Treatment of Ebola Virus Infected Nonhuman Primates with Hyperimmune Equine Immunoglobulin Fragments

Xiangguo Qiu, M.D.[1], Shihua He, Ph.D.[1], Wenjun Zhu, Ph.D.[1], Gary Wong, Ph.D.[1], Hualei Wang, Ph.D.[2], Yongkun Zhao, Ph.D.[2], Feihu Yan, M.S.[1], Songtao Yang, Ph.D.[2], Xlanzhu Xia, B.S.[2]
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135. A-to-I Editing by ADAR1 Limits HPV Expression by Regulating Innate Immunity

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136. NonInvasive Topical Immunization Using Cholera Toxin As Adjuvant for the Treatment of Hepatitis B

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137. Characterization of Proteases of a Clade-D Betacoronavirus

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138. Development of Cholesterol-conjugated Stapled Peptides as Inhibitors of EBOLA Virus Infection

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139. Hijacking of PI4KB by Picornaviruses – Structural Insights and the Role of ACBD3

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140. Phenotypic Screening Funnel for Evaluation of Therapies against BSL-3 Neurotropic Alphaviruses

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141. Nano-delivery for Drugs with Antiviral Properties Based on BSA as a Carrier

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142. Screening of an FDA-approved Compound Library Targeting the mRNA Capping of Venezuelan Equine Encephalitis Virus (VEEV)

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144. Acetohydroxamic Metal-Chelators against Hepatitis C Virus and Flaviviruses

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145. Inhibition of Coronaviruses by Beta-D-N4-hydroxycytidine (NHC)

Maria Agostini, B.S.[1], Erica Andres, B.S.[1], James Chappell, M.D., Ph.D.[1], Amy Sims, Ph.D.[2], Rachel Graham, Ph.D.[2], Timothy Sheahan, Ph.D.[2], Michael Natchus, Ph.D.[3], Ralph Baric, Ph.D.[2], George Painter, Ph.D.[3], Mark Denison, M.D.[1]
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146. SAMHD1 is a Modulator of Nucleos(t)ide Analogues Efficacy

Marc Castellví, M.S.[1], Eudald Felip, M.D.[2], Maria Pujantell, M.S.[1], Edurne Garcia-Vidal, M.S.[1], Eva Riveira-Muñoz, Ph.D.[1], Roger Badia, Ph.D.[1], Bonaventura Clotet, M.D., Ph.D.[1], Mireia Margelí, M.D., Ph.D.[2], José Esté, Ph.D.[1], Ester Ballana, Ph.D.[1]
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147. Low Fidelity of SARS-Coronaviruses Polymerase Complex

Nhung Le, M.S.[1], Lorenzo Subissi, Ph.D.[1], Ana Theresa Morais, Ph.D.[1], Isabelle Imbert, Ph.D.[1], Bruno Canard, Ph.D.[1]
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148. Human Parainfluenza-3 Inhibition by Nucleoside Analogues in a Human Airway Epithelium Cell Model.

Auke De Zwart, M.D.[1], Steven De Jonghe, Ph.D.[2], Jan-Willem Alffenaar, Ph.D.[1], Erik Verschuuren, M.D., Ph.D.[1], Wolfgang Pfleiderer, Ph.D.[3], Piet Herdewyn, Ph.D.[2], Annelies Riezebos-Brilman, M.D., Ph.D.[1], Johan Neyts, Ph.D.[2], Dirk Jochmans, Ph.D.[2]
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149. Negative Charge and Membrane Tethered Viral 3B Cooperate to Recruit Viral RNA-Dependent RNA Polymerase $3D^{pol}$.

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150. Use of a Dual Luciferase Cell-Based Drug Screening Assay to Study VP24 Inhibition of JAK/STAT Pathway and its Reversion by Compounds

Elisa Fanunza, M.S.[1], Aldo Frau, M.S.[1], Marco Sgarbanti, Ph.D.[2], Roberto Orsatti, Ph.D.[2], Angela Corona, Ph.D.[1], Enzo Tramontano, Ph.D.[1]
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151. Favipiravir-resistant Chikungunya Virus is Severely Attenuated in Mosquitoes

Leen Delang, Ph.D.[1], Sofie Jacobs, M.S.[1], Rana Abdelnabi, Ph.D.[1], Pei-Shi Yen, Ph.D.[2], Anna-Bella Failloux, Ph.D.[2], Johan Neyts, Ph.D.[1]
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152. New Antivirals for Bovine Viral Diarrhea Virus Selected by Virtual Screening against Viral RNA Polymerase

Maria España, B.S.[1], Matias Fabiani, B.S.[2], Juan Casal, Ph.D.[3], Mariela Bollini, Ph.D.[3], Lucia Cavallaro, Ph.D.[1], **Eliana Castro, Ph.D.**[2]
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153. Direct Cell-To-Cell Spread can be Specifically Targeted by Small Molecule Inhibitors.

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155. Targeting Histone Deacetylation: Effects On Host Inflammation and Lung Pathology In Influenza A/H3N2-Infected Mice

Lora Simeonova, Ph.D.[1], Galina Gegova, M.S.[1], Petya Dimitrova, Ph.D.[2]
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156. Antiviral Efficacy Of Tilorone Dihydrochloride against Bunya- And Paramyxoviruses

Kendra Johnson, B.S.[1], Birte Kalveram, Ph.D.[1], Colm Atkins, Ph.D.[1], Lihong Zhang, M.D.[1], **Alexander Freiberg, Ph.D.**[1]
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157. Crystallography-Based Drug-Like Fragment Screening with Polyomavirus VP1

Ali Munawar, M.S.[1], Steven Beelen, B.S.[1], Stephen Weeks, Ph.D.[1], **Sergei Strelkov, Ph.D.**[1]
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158. Ouabain Inhibits Influenza A Viruses Replication

Jang-Hoon Choi, Ph.D.[1], Junhyung Cho, M.S.[1], Eun Young Jang, M.S.[1], Mi-Seon Lee, M.S.[1], Kisoon Kim, Ph.D.[1]
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159. Novel Diphosphate Analogues of PMEA: Synthesis and Study of their Ability to Inhibit HIV-RT

Wolfgang Laux, Ph.D.[1], Stéphane Priet, Ph.D.[2], Christian Périgaud, Ph.D.[1], Karine Alvarez, Ph.D.[3], Suzanne Peyrottes, Ph.D.[1]
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160. Potent and Broad-Spectrum Small Molecules that Disrupt the PA-PB1 Subunits Interaction of Influenza Virus RNA Polymerase and Possess a High Genetic Barrier to Drug-Resistance

Giulio Nannetti, Ph.D.[1], Beatrice Mercorelli, Ph.D.[1], Serena Massari, Ph.D.[2], Jenny Desantis, Ph.D.[2], Laura Goracci, Ph.D.[3], Paul Digard, Ph.D.[4], Gabriele Cruciani, Ph.D.[3], Oriana Tabarrini, Ph.D.[2], Giorgio Palù, M.D.[1], **Arianna Loregian, Ph.D.**[1]
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161. Combinations of Terminase Inhibitors Elicit a Synergistic Enhancement of Effect against Human Cytomegalovirus in vitro

Kylie Markovich, B.S.[1], Brian Gentry, Ph.D.[1]
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162. The Inhibitory Effect of Mycophenolic Acid Derivatives on MERS Coronavirus Papain-Like Protease

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163. Env-Driven Replicative Fitness Seems to be Associated with a Reduced Response to cART in Patients Infected with HIV-1 Subtype F Strains

ME Quinones-Mateu, Ph.D.[1], D Winner, B.S.[1], S Joussef-Pina, Ph.D.[1], B Pernas, M.D.[2], A Aguilera, M.D.[3], E Poveda, Ph.D.[2]
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165. Disrupting Viral Transcriptional Regulatory Circuitry Constitutes an Escape Resistant Therapeutic Strategy

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166. Mapping the Mutations in HCV Protease Conferring Resistance to Grazoprevir or Danoprevir

Guigen Zhang, Ph.D.[1], Qiheng Li, B.S.[1], Wensheng Wei, Ph.D.[1]
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167. A Drug Repurposing Approach Identifies Different Approved Compounds that Specifically Inhibit Human Cytomegalovirus (HCMV) Replication with Mechanisms Different from that of the Current Anti-HCMV Drugs

Beatrice Mercorelli, Ph.D.[1], Anna Luganini, Ph.D.[2], Marta Celegato, Ph.D.[3], Giulio Nannetti, Ph.D.[3], Giorgio Palù, M.D.[3], Giorgio Gribaudo, Ph.D.[2], Arianna Loregian, Ph.D.[3]
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168. CD32 Expression is Associated to T Cell Activation and Upregulated by HIV

Roger Badia, Ph.D.[1], Ester Ballana, Ph.D.[1], Marc Castellví, B.S.[1], Edurne García-Vidal, B.S.[1], Maria Pujantell, B.S.[1], Bonaventura Clotet, M.D., Ph.D.[1], Miguel Angel Martínez, Ph.D.[1], Eva Riveira-Muñoz, Ph.D.[1], José A. Esté, Ph.D.[1]
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169. Synthesis of Non-Hydrolysable "Super Substrate" of Phosphatidylinositol 4-Kinases, Important Players in Host-Virus Interaction

Radim Nencka, Ph.D.[1], Hubert Hřebabecký, Ph.D.[1], Eliška Procházková, Ph.D.[1], Eva Zborníková, M.S.[1], Milan Dejmek, Ph.D.[1]
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170. Anti-Varicella Zoster Virus Activity of Amenamevir for Treatment of Herpes Zoster

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171. Indolylarylsulfones with Potent Anti-HIV-1 Activity

Domiziana Masci, Ph.D.[1], Giuseppe La Regina, Ph.D.[1], Antonio Coluccia, Ph.D.[1], Andrea Brancale, Ph.D.[2], José Esté, Ph.D.[3], Romano Silvestri[1]
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172. Metabolic Activation of MBX-2168 Involves Enzymatic Removal of a Butyl-Ether Moiety by Adenosine Deaminase-Like Protein 1

Anna Burns, B.S.[1], Hannah Sauer, B.S.[1], John Williams, Ph.D.[2], Marc Busch, Ph.D.[3], Terry Bowlin, Ph.D.[2], Brian Gentry, Ph.D.[1]
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174. Biological Evaluation of Novel Small-Molecule Antiviral Agents versus Tick Borne Encephalitis Virus

Friederike Hucke, D.V.M.[1], Daniela Friese, B.S.[1], Adriana Helfen, B.S.[1], Marcella Bassetto, Ph.D.[2], Andrea Brancale, Ph.D.[2], **Joachim Bugert, M.D., Ph.D**.[1]
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175. Antiviral Synergy between Zika Virus Protease and Polymerase Inhibitors

Jasper Chan, M.D.[1], Kenn Chik, M.S.[2], Shuofeng Yuan, Ph.D.[2], Cyril Yip, Ph.D.[2], Vincent Poon, M.S.[2], Chris Chan, M.S.[2], Kwok-Yung Yuen, M.D.[1]
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176. Identification of Combinations of Approved Drugs with Synergistic Activity against Ebola Virus in Cell Cultures

Julie Dyall, Ph.D.[1], Elizabeth Nelson, Ph.D.[2], Lisa Dewald, Ph.D.[1], Rajarshi Guha, Ph.D.[3], Brit Hart, M.S.[1], Hunaying Zhou, M.S.[1], Elena Postnikova, M.S.[1], James Logue, B.S.[1], Walter Vargas, B.S.[1], Robin Gross, B.S.[1], Julia Michelotti, Ph.D.[1], Nicole Deiuliis, M.S.[1], Richard Bennett, Ph.D.[1], Ian Crozier, M.D.[1], Michael Holbrook, Ph.D.[1], Patrick Morris, Ph.D.[3], Carleen Klumpp-Thomas, Ph.D.[3], Crystal McKinght, Ph.D.[3], Paul Shinn, Ph.D.[3], Pamela Glass, Ph.D.[4], Lisa Johansen, Ph.D.[5], Peter Jahrling, Ph.D.[1], Lisa Hensley, Ph.D.[1], Gene Olinger, Ph.D.[1], Craig Thonas, Ph.D.[3], Judith Whtie[2]
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177. Metal Chelating Agents against Viruses

Grigoris Zoidis, Ph.D.[1], Erofili Giannakopoulou, M.S.[1], Vasiliki Pardali, M.S.[1], Tiffany Edwards, M.S.[2], Efseveia Frakolaki, M.S.[3], Vassilios Myrianthopoulos, Ph.D.[1], Emmanuel Mikros, Ph.D.[1], Ralf Bartenschlager, Ph.D.[4], Niki Vassilaki, Ph.D.[3], John Tavis, M.D., Ph.D.[2]
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178. Mapping Influenza Hemagglutinin Evolutionary Conservation to its Structural Plasticity: When sequence and structural dynamics can elucidate conserved epitope accessibility

Marion Sauer, B.S.[1], Jens Meiler, Ph.D.[1], James Crowe, M.D.[1]
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179. Nanobodies Reveal Functional Epitopes and Potential Mechanisms of Norovirus Neutralization

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180. *N*[4]-*p*-nitrophenylthiosemicarbazone of 5,6-dimethoxy-1-indanone as a New Non Nucleoside Inhibitor of BVDV RNA Polymerase

Matías Fabiani, B.S.[1], Eliana Castro, Ph.D.[1], María Quintana, B.S.[2], María Soraires Santacruz, B.S.[3], María España De Marco, B.S.[4], Graciela Moltrasio, Ph.D.[5], Liliana Finkielsztein, Ph.D.[6], Alejandra Capozzo, Ph.D.[7], Lucía Cavallaro, Ph.D.[8]
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181. Hepatitis B Virus Core Protein Dephosphorylation is Required for Pregenomic RNA Encapsidation

Qiong Zhao, Ph.D.[1], Junjun Cheng, Ph.D.[1], Shuo Wu, Ph.D.[1], Yue Luo, M.D.[1], Jinhong Chang, Ph.D.[1], **Ju-Tao Guo, M.D.**[1]
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182. Development & Characterization of Poly ξ-Caprolactone Nanoparticles for Vaccine Delivery

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183. Studies on the Mechanism of Bacteriophage Mediated Inhibition of Human Adenovirus Type 5 Infection *In Vitro*

Maciej Przybylski, Ph.D.[1], Renata Jakubowska-Zahorska, M.D., Ph.D.[1], Ryszard Międzybrodzki, M.D., Ph.D.[2], Jan Borysowski, M.D., Ph.D.[3], Beata Weber-Dąbrowska, Ph.D.[2], Tomasz Dzieciątkowski, Ph.D.[1], Andrzej Górski, M.D., Ph.D.[3]
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184. Influenza A/H3N2 Virus Infection in Immunocompromised Ferrets and Emergence of Antiviral Resistance

Rueshandra Roosenhoff, M.S.[1], Erhard van der Vries, Ph.D.[2], Anne van der Linden, B.S.[1], Geert van Amerongen, B.S.[3], Koert Stittelaar, Ph.D.[3], Saskia Smits, Ph.D.[3], Martin Schutten, Ph.D.[4], Ron A.M. Fouchier, Ph.D.[1]
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185. A Live-Attenuated HSV-1 VC2 Vaccine Protects against HSV-2 Genital Infection in Guinea Pigs

Rhonda Cardin, Ph.D.[1], Fernando Bravo, M.D.[1], Brent Stanfield, Ph.D.[2], Derek Pullum, B.S.[1], David Dixon, M.S.[1], Vladimir Chouljenko, Ph.D.[2], Konstantin Kousoulas, Ph.D.[2], David Bernstein, M.D.[1]
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189. Development of Engineered Nanocarrier for Controlled Delivery of a Protease Inhibitor

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191. *In Situ* Imaging of High Consequence Pathogens in Rodents using Fluorescent Reporter Viruses

Jessica Spengler, D.V.M., Ph.D.[1], Stephen Welch, Ph.D.[1], Michael Lo, Ph.D.[1], Jana Ritter, D.V.M.[2], JoAnn Coleman-McCray, B.S.[1], Florine Scholte, Ph.D.[1], Jessica Harmon, M.S.[1], Éric Bergeron, Ph.D.[1], Sherif Zaki, M.D., Ph.D.[2], Stuart Nichol, Ph.D.[1], Christina Spiropoulou, Ph.D.[1]
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193. Liver Targeted Delivery of Clevudine-5′-Monophosphate Reduces Systemic Clevudine Exposure in Rats: Implications for the Treatment of HBV Infections

David Guthrie, Ph.D.[1], **Gregory Bluemling, Ph.D.**[2], Manohar Saindane, Ph.D.[1], Zachary Sticher, B.S.[1], Vindhya Edpuganti, Ph.D.[1], Deborah Mitchell, B.S.[1], Michael Natchus, Ph.D.[1], George Painter, Ph.D.[3], Alexander Kolykhalov, Ph.D.[2], Abel De La Rosa, Ph.D.[2]
[1]*Emory Institute for Drug Development;* [2]*Emory Institute for Drug Development, DRIVE;* [3]*Emory Institute for Drug Development, DRIVE, Emory Department of Pharmacology*

250. USC-087, an HPMPA Prodrug, Prevents Varicella Zoster Virus Replication in Human Skin in Culture and in a Mouse Model

Jennifer Moffat, Ph.D.[1], Dongmei Liu, Ph.D.[1], Mark Prichard, Ph.D.[2], Jiajun Fan, Ph.D.[3], Jinglei Lyu, Ph.D.[3], Boris A. Kashemirov, Ph.D.[3], and Charles E. McKenna, Ph.D.[3]

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252. A Novel Flavivirus Inhibitor, DVI-1, Discovered Through High-Throughput Screening (HTS) with Dengue Reporter Viruses

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Abstracts

1. Antiviral Investigation, High Performance Liquid Chromatography Analysis and Phytochemical Profiling of Bryophyllum pinnatum and *Viscum album*

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BACKGROUND: Despite tremendous progress in human medicine, no drugs exist for the treatment of measles, polio, yellow fever and herpes simplex-1. Aim: This study was designed to investigate the antiviral potentials of two medicinal plants available locally in South Eastern, Nigeria. Materials and Methods: Fresh leaves of *Bryophyllum pinnatum* and Viscum album were collected from Owerri, South Eastern Nigeria. Extraction of the plant materials was done with methanol using the soxhlet extractor and concentrated using the rotary evaporator. The plant materials were allowed to dry and after ascertaining their toxicity profile, they were subjected to antiviral analysis using 100$TCID_{50}$ of measles, polio, yellow fever and herpes simplex-1 viruses. Results: The toxicity profile shows that the minimum non-toxic concentration (MNTC) of *B. pinnatum* was 0.016 µg $µL^{-1}$ with an IC_{50} of 0.313 µg $µL^{-1}$ while that of *V. album* was 0.063µg $µL^{-1}$ and IC_{50} of 0.063µg $µL^{-1}$. Result of the antiviral analysis shows that *B. pinnatum* and *V. album* produced activity against measles virus and herpes simplex virus – 1 at the concentrations of 0.016 µg $µL^{-1}$ (IC_{50} 0.004 µg $µL^{-1}$; IC_{50} 0.004 µg $µL^{-1}$) and 0.063µg $µL^{-1}$ (IC_{50} 0.031 µg $µL^{-1}$; IC_{50} 0.039 µg $µL^{-1}$) respectively, while polio and yellow fever viruses were resistant to both plants extracts at all the concentration tested. Result of the phytochemical analysis revealed the presence of various secondary metabolites. CONCLUSION: This study has shown that the solution to measles and herpes simplex-1 viral diseases could be found in the forest zones of Nigeria.

2. Susceptibility of Paramyxoviruses and Filoviruses to Inhibition by 2α-Monofluoro- and 2α-Difluoro-4α-Azidocytidine Analogs

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Ebolaviruses, marburgviruses, and henipaviruses are zoonotic pathogens belonging to the *Filoviridae* and *Paramyxoviridae* families. They exemplify viruses that continue to spill over into the human population, causing outbreaks characterized by high mortality and significant clinical sequelae in survivors of infection. There are currently no approved small molecule therapeutics for use in humans against these viruses. In this study, we evaluated the antiviral activity of the nucleoside analog 4ř-azidocytidine (4řN_3-C, R1479) and its 2ř-monofluoro- and 2ř-difluoro-modified analogs (2řF-4řN_3-C and 2řdiF-4řN_3-C) against representative paramyxoviruses (Nipah virus, Hendra virus, measles virus, and human parainfluenza virus 3) and filoviruses (Ebola virus, Sudan virus, and Ravn virus). We observed enhanced antiviral activity against paramyxoviruses with both 2řdiF-4řN_3-C and 2řF-4řN_3-C compared to R1479. On the other hand, while R1479 and 2řdiF-4řN_3-C inhibited filoviruses similarly to paramyxoviruses, we observed 10-fold lower filovirus inhibition by 2řF-4řN_3-C. To our knowledge, this is the first study to compare the susceptibility of paramyxoviruses and filoviruses to R1479 and its 2ř-fluoro-modified analogs. The activity of these compounds against negative-strand RNA viruses endorses the development of 4ř-modified nucleoside analogs as broad-spectrum therapeutics against zoonotic viruses of public health importance.

3. Several Mechanisms Contribute to Photodynamic Inhibition of HSV-1 Infection

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Photodynamic therapy (PDT) is widely used to treat different tumors and is rapidly developing approach to inhibit replication of fungi, bacteria and viruses. Herpes simplex virus 1 (HSV-1) is frequently used model to study effects of PDT on enveloped viruses. Although a number of photosensitizers (PS) has been tested for the antiviral properties, the analyses are usually limited to assessing the reduction in virus yield, and thus the molecular mechanisms of photodynamic inactivation remain poorly understood. In our study, we investigated the antiviral properties of TMPyP3-$C_{17}H_{35}$, an amphiphilic porphyrin-based PS, and the molecular mechanisms of HSV-1 inhibition. We show that the expression of genes (immediate early, early and late genes) was strongly reduced in cells pretreated with sub-toxic concentrations of TMPyP3-$C_{17}H_{35}$, leading to a significantly reduced virus replication. These results indicate that treatment of cells with the compound triggers unexplored intrinsic antiviral defense mechanisms. Curiously, in experiments where cells were treated after the infection we observed a strong effect of TMPyP3-$C_{17}H_{35}$ on the virus yield only when cells were treated shortly (30min) after the infection. There are several plausible explanations for such effect, however, inefficiency of the compound to inhibit the virus once it has initiated its transcription, indicates incoming virions as the major target, probably by damaging internalized virus capsids, disrupting internalization to nucleus and at the same time triggering antiviral intrinsic immunity. Indeed, we show that the compound dramatically decreases free-virus infectivity. Taken together, our results show that activated TMPyP3-$C_{17}H_{35}$ effectively inhibits HSV-1 replication by several different mechanisms.

5. Screening and Distribution of Different Genes Related to Cytoskeleton and Neuronal Transmitters in Hippocampus under Rabies Infection

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The objective of the present study was to illuminate the findings of neuronal dysfunction associated with rabies virus (RABV) infection by observing genes related to neurotransmitters and cytoskeleton. In addition, the distributions of rabies glycoprotein antigens in different areas of brain tissues were also observed through immunohistochemistry. Real time PCR and immunohistochemistry were employed to interpret the difference in the signal peptide sequence of amino acid among fixed strains (3aG, CVS) and street (SX, PB3) strains of RABV. Moreover, the neuronal structures of hippocampus were also observed under electron microscope.

The results demonstrated full similarity between SX and PB3 strains, while 84.2% similarity of signal peptide exist between CVS and 3aG. It is well known fact that stathmin1 (stmn1), katna1, adenomatous polyposis coli (APC) control neuronal cell differentiation, while glycogen synthase kinase 3ř (gsk3ř) regulate stability of intracellular microtubules; myosin light chain kinase (mylk) regulates intracellular filamentous actin; kinesin family member 5B (kif5) and kinase light chain 1 (klc1) hold the responsibility of transport events involving neuronal factors and vesicles, while mylk regulates actins and releasing of neurotransmitters. An up-regulation in genes expression of katna1, apc, gsk3ř, mlck and gelsolin was observed, while down-regulated expression was seen for klc1 and Stmn1. The order of glycoprotein antigens were highest in cerebral cortex followed by hippocampus, brain stem, cerebellum, thalamus and olfactorybulb which was consistent with RABV distribution observed through immunohistochemistry. The electron microscopy demonstrated swelling of mitochondrion, disappearance in mitochondrial cristaes, hyper function of golgi complex and expansion of perinuclear cisterna.

6. Inactivation Effects of Calcium Hydrogen Carbonate Mesoscopic Crystals On Enveloped or Non-Enveloped Animal DNA and RNA Viruses.

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Virucidal effects of a novel electrically charged disinfectant, CAC-717, on various kinds of viruses were investigated. CAC-717 is an alkaline solution (pH 12.3) and has a self-electromotive force, but it is harmless and not irritant to humans and animals because it contains no chemicals. CAC-717 is produced by applying an electric field to mineral water containing calcium hydrogen carbonate in order to generate mesoscopic crystals.

Four kinds of animal virus combinations were used: enveloped DNA viruses (herpesvirus and poxvirus), non-enveloped DNA viruses (parvovirus and adenovirus), enveloped RNA viruses (orthomyxovirus, paramyxovirus, coronavirus and flavivirus), and non-enveloped RNA viruses (picornavirus and reovirus). Nine volumes of CAC-717 were mixed with one volume of virus and incubated for 1 hour at room temperature. Virus titers were determined by titration in appropriate cell cultures. Effects of CAC-717 on viral genomes in the virions were examined by quantifying their genomes by real-time PCR.

Virus titration analyses showed that CAC-717 treatment achieved reductions of 2 log10 to 5.5 log10 or more against all of the viruses tested. Real-time PCR revealed that genomes of RNA viruses except for flavivirus were reduced drastically after CAC-717 treatment.

Virucidal effects of CAC-717 on enveloped or non-enveloped DNA and RNA viruses were observed. The electrical potential generated on the surfaces of minerals in CAC-717 and its high pH value (pH 12.3) might play key roles in the anti-viral mechanism of CAC-717. CAC-717 may be used effectively and safely as a disinfectant against various kinds of animal viruses.

7. Identification of Compounds with Effect On Cell Proliferation, Apoptosis and Viral Tax Expression in HTLV-1-Infected Cell Line

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INTRODUCTION. Currently, there is no cure and no vaccine for human T cell lymphotropic virus type 1 (HTLV-1) infection. Therapeutic approaches for the main HTLV-1-associated diseases (HTLV-1 associated myelopathy/tropical spastic paraparesis-HAM/TSP, and adult T cell leukemia/lymphoma-ATL) have variable effectiveness. Thus, identification of effective antiviral compounds on infected cells is required. Aim. To screen 26 synthetic compounds for identification of cell proliferation inhibitors and apoptosis inducers in HTLV-1-infected cell line (MT-2), and to assess the drug effect on viral tax expression through inducible-tax reporter cell. Methods. MT-2 cells were plated with 26 compounds, which were synthesized by Organic Synthesis. These compounds were serially diluted by factor of 2 and a resazurin reduction method was adopted. EC_{50} of each compound was also determined. Activity of selected compounds was confirmed by cell cycle analysis, apoptosis assays (caspase-3/7 and annexin/PI) and tax/GFP expression analysis through flow cytometry. Results. Regarding the 26 compounds screened, eight presented activity ≥ 70% at 50 μM in at least two assays, of which three compounds showed interesting EC_{50} values (14.0 ± 5.48; 10.0 ± 0; 9.46 ± 6.70 μM). Moreover, compound 61 was the only one that induced an S phase cell cycle arrest and compounds 48, 49, 65 and 66 promoted apoptosis. Compounds 48 and 49 were also able to reduce GFP expression in inducible-tax reporter cell, which suggests an effect on viral protein. CONCLUSION. Our data suggest that these synthetic compounds should be investigated as possible novel drugs for treatment of HTLV-1 infection and the associated diseases.

8. Comparison of Two Methods for Igm Anti-HAV Antibodies Measurement

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INTRODUCTION: IgM anti-HAV antibodies mean a recent infection with hepatitis A virus. IgM anti-HAV antibodies generally can be detected in the blood as early as 2 weeks after the initial HAV infection.

MATERIALS AND METHODS: The study included 100 samples for determination of IgM anti-HAV using Architect (Abbott) and Cobas (Roche). HAV IgM was performed on the same day on both assays. The Architect signal-to-cutoff (S/CO) values of < 0.80 S/CO was on reactive; 0.80-1.20 was considered gray-zone values; reactive was > 1.20. The Cobas reference range was non reactive < 0.7 COI; reactive ≥ 1.0 COI; 0.7-1.0 gray-zone values.

RESULTS: The 100 samples were measured, 45 (45%) were reactive and 50 (50 %) were nonreactive for both kits. Among the 5 (5 %) discrepant serum, showed gray-zone values with Architect, but they were nonreactive in Cobas. The patients with early phase of HAV infection, the Architect showed slightly later seroconversions compared to the Elecsys. In two samplers using Architect we got nonreactive in one sample and gray-zone in another, suggesting a slight difference in the sensitivity for the detection of decreasing anti-HAV IgM in patients who had recovered from previous HAV infection. The correlation of assays was doe using Pearson's correlation coefficient (r) between Architect and Cobas 0.69 (P<0.001). The accuracy was 0.079-5.56 % CV for both assays.

COCLUSIONS: The two automated immunoassay kits showed comparable performances, with excellent overall agreement when they have been used on patients samplers and can be successfully applied in clinical laboratory practice.

9. ACBD3, a Pan-Enterovirus Host Factor That Scaffolds Enterovirus 3A Proteins and the Host Factor PI4KB

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The *Enterovirus* genus of the *Picornaviridae* family includes many important human pathogens such as enterovirus-A71, poliovirus, coxsackievirus, and rhinovirus. Still, there are currently no antiviral drugs, and vaccines are only available against poliovirus and enterovirus-A71. Like all +RNA viruses, enteroviruses induce reorganization of host cell membranes to form replication organelles, which is required for efficient viral genome replication. Phosphatidylinositol 4-kinase IIIř (PI4KB) is an essential host factor utilized by all enteroviruses. Enterovirus 3A protein recruits PI4KB to the replication organelles, yet the underlying molecular mechanism remains elusive. In this study, we investigated the role of ACBD3 in enterovirus replication and the recruitment of PI4KB using ACBD3-knockout cells. Representative members of six human enterovirus species showed deficient growth kinetics in ACBD3-knockout cells. In the absence of ACBD3, PI4KB could not be recruited either in virus-infected cells or in cells expressing viral 3A individually. The lack of ACBD3 also caused a relocalization of 3A from the Golgi to the endoplasmic reticulum. Reconstitution of wildtype ACBD3 restored 3A localization, PI4KB recruitment, and virus replication. In contrast, an ACBD3 mutant that cannot interact with PI4KB could restore the localization of 3A but could not recruit PI4KB or support efficient virus replication. This study implicates that ACBD3 plays a key role in enterovirus replication as a scaffold that organizes both viral and host proteins. Compounds that target ACBD3 or the 3A-ACBD3 interaction could be a novel strategy for the development of broad-acting anti-enteroviral compounds.

10. Eukariotic Ddx3 Helicase, a Potential Target for Virus Treatment

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DDx3 helicase belongs to the family of DEAE-box and play a key role in RNA metabolism. A role of DDx3 helicase in HIV life cycle has been proposed as a shuttling protein responsible for the export of unspliced/partialy spliced HIV RNAs from nucleus to cytoplasm. The knockdown of DDx3 is associated with the inhibition of HIV replication. DDx3 also play a key role in the replication of Dengue, West-Nile, Hepatitis C, and Japanese Encephalitis viruses. On this basis, DDx3 helicase can be conceded as a target for developing DDx3 inhibitors to block HIV replication. Herein, we present data on expression, purification and some properties of human DDx3 helicase. Based on diarylurea-, triazolotriazine- and pyrimidinacetamide derivatives we synthesized 43 novel compounds as inhibitors of ATPase and helicase activities of DDx3. Several compounds displayed also the activity against HIV and the tick-borne encephalitis virus replication in cell cultures.

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11. The Determination of Antimicrobial Sensitivity On Resistance of Bacteria

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INTRODUCTION: The resistance problem occurs as a result of the adaptation of microorganisms to antibiotics and as a result of inadequate dosing and duration of therapy.

MATERIALS AND METHODS: An analysis of antimicrobial sensitivity/bacterial resistance assay was performed. The isolates from these are also identified and their antimicrobial sensitivity/resistance was determined by standard microbiological testing methods.

RESULTS: During this study, in the total sample, the most common isolates are enterobacteria, represented by 94% in urine samples and 51.6% in wound samples. E. coli was present in 82% isolates in urine samples and 30.5% in wound isolates. The wound samplers were dominance of Staph. aureus was emphasized. The highest resistance to gram negative bacteria was a*mpicillin* showed E. coli and Klebsiella spp from 82% to 100%. Resistance to c*efuroxime* showed the highest Proteus spp from 44% to 50%. The major resistance to ciprofloxacin also showed Proteus spp. from 47% to 51%. Pseudomonas spp. showed the highest rate of resistance to gentamicin, from 66.7 to 70%. The highest resistance to c*o-trimoxazole* was E. coli up to 60% and Proteus spp. from 67% to 70%. Staph. aureus has high resistance to penicillin from 95% to 98%. Enterococcus faecalis showed the highest resistance to gentamicin up to 62.5% and to ciprofloxacin up to 48%.

CONCLUSION: Excessive use of antibiotics, especially in situations where their use is not justified, is the most important reason for the development of resistance to antibiotics and is one of the key public health problems.

12. Repurposing Heparin and Its Derivatives to Prevent ZIKA Virus-Induced Cell Death in Human Three-Dimensional (3D) Neurospheres (NS) Derived From Pluripotent Stem Cells

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The severe consequences of fetal ZIKA virus (ZIKV) infection have highlighted the need of antiviral agents for the treatment of infected pregnant women. One attractive approach for combating emerging and rapidly spreading infectious diseases is drug repurposing. Heparin, a widely used anticoagulant safely used in pregnant women, has antiviral activities against attachment and entry of several enveloped viruses. The aim of the study is to investigate the effects of heparin and its derivatives on ZIKV replication and cytopathic effects (CPE) in human neural progenitor cells (NPCs) grown as neuropheres (NS). Incubation of NPCs with heparin (100 µg/ml) 1 h prior to ZIKV infection resulted in a significantly preservation of the NS diameter (513±26 *vs.* 241±11 µm in uninfected and infected NS, respectively). A significant increase of AK activity was determined in infected cells whereas heparin decreased it to the levels observed in uninfected cells. Moreover, heparin prevented the formation of intracellular vacuoles, a typical feature of paraptosis, together with the inhibition of HMGB1 release in NS culture supernatant. Structure and function analysis indicated that heparin sulfation is dispensable for inhibiting ZIKV-induced CPE whereas fractions devoid of anticoagulant activity were also effective in inhibiting ZIKV-induced CPE. The mechanism underneath this prevention of viral-induced cell death by heparin will need further investigations. In summary, heparin and its desulphated derivatives could be potentially exploited as lead compounds to discover novel agents for preventing virus replication and CPE.

13. The Value Ferritin and Lactate in CSF in Patients with Meningitis

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INTRODUCTION: The aim of our study was detection of the value of ferritin and lactate in CSF (cerebrospinal fluid) at patients with meningitis.

MATERIALS AND METHODS: In the period of 2016-2017, we determined CSF from 100 patients (69 cases diagnosed with bacterial meningitis and 31 patients as no-meningitis). The diagnostic criteria of bacterial meningitis included presence of more than five leukocytes in cubic millimeters of cerebrospinal fluid with dominance of polymorphonuclear cells, high lactate low glucose and high protein in cerebrospinal fluid. Cerebrospinal fluid ferritin was measured by chemiluminescence assay on ARCHITECT I 2000 SR (ABBOTT). Lactate was measured using dry chemistry of VITROS 5600 analyser.

RESULTS: The patients with bacterial meningitis have a value of ferritin in liquor 46.7 +/- 5.3 ng/ml and lactate 4.7 +/- 1.3 mmol/L. The protein in CSF was determined and in patients with bacterial meningitis with value 4.2 g/L and

glucose was 1.3 mmol/L. On no- meningitis patients in CSF we got ferritin in liquor 12 +/- 2.2 ng/ml and lactate 1.4+/- 0.3 mmol/L. The protein in CSF in patients with no-meningitis and the value were 0.20 +/-0.10 and glucose 2.5+/-1.0 mmol/L. Using *Mann–Whitney* U test it was found statistic significant differences for $p < 0.05$. The number leukocytes have a good correlation with ferritin r = 0.91

CONCLUSION: The ferritin is the maker of the acute phase and hypoxia therefore could be used as a diagnostic parameter in meningitis. If leukocytes and ferritin increase than glucose fall down.

14. Identification of Novel Interferon Gamma-Induced, Anti-Retrovirus Host Factors

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Interferons (IFNs) are cytokines that have anti-virus activity. IFNs are divided into 3 types according to their receptors. When cells detect viruses, they express type I IFN. Thus, mechanism of the anti-virus activity of type I IFN (IFN-alpha or -beta) has been vigorously studied, and several anti-virus host factors induced by the type I IFN have been already identified. However, the mechanism by which type II IFN (IFN-gamma) restricts virus replication is still unknown. Recently, we have reported that Gamma-IFN-inducible Lysosomal Thiolreductase (GILT) significantly inhibits single round infection mediated by envelope glycoprotein (Env) of HIV-1 (Kubo et al. Oncotarget (2016) 7:71255-71273). In HeLa cells, however, GILT silencing did not affect the anti-virus activity of IFN-gamma, showing that other anti-virus host factors are induced by IFN-gamma in HeLa cells. To identify such factors, microarray analysis of untreated and IFN-gamma-treated HeLa cells was performed. Already known host restriction factors were not induced by IFN-gamma. cDNA clones of host factors that were elevated more that 10 times by IFN-gamma were isolated, and HeLa cells stably expressing the factors were constructed. Transduction titers of HIV-1 Env-containing HIV-1 vector were measured in these cells. HeLa cells expressing shRNA against these factors were also constructed, and transduction titers were measured in presence or absence of IFN-gamma. As the result, three novel anti-retrovirus factors were identified. We will talk the mechanisms of these host restriction factors to inhibit HIV-1 Env-mediated infection.

15. Chikungunya Virus Nsp3 Associates with the Lipid Kinase Sphingosine Kinase 2

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We have previously shown that the lipid kinase sphingosine kinase 2 (SK2) is recruited to the CHIKV replication complex (vRC). In the current study we reveal that the nsP3 protein recruits SK2 through an association with its hypervariable domain(HVD).

16. Heteroarylpyrimidine (HAP) and Novel Non-HAP Capsid Assembly Modifiers Show Differences in their Mode of Action *In Vitro*

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In the search for Hepatitis B Virus (HBV) cure, the capsid is one of the most promising virus-encoded targets, since its basic subunit, the core protein (HBcAg), is essential for several steps in the viral life cycle, including synthesis of the viral genome, production of infectious virions, and maintenance of the covalently closed circular DNA (cccDNA) pool in the nucleus. This study investigated differences in the mode of action and antiviral activity of six different capsid assembly modulator (CAM) scaffolds: heteroarylpyrimidine, sulfamoylbenzamide, phenylpropenamide, glyoxamide-pyrrolamide, pyrazolyl-thiazole, and dibenzo-thiazepin-2-one. The representative CAMs tested yielded different potencies in biochemical target assays and cell-based antiviral assays. Analytical size exclusion chromatography and native agarose gel electrophoresis showed that at higher CAM concentrations, only HAP produced aggregates and structures larger than the expected capsid size. Consequently, aberrant non-capsid polymers were only seen in transmission electron microscopy after HAP treatment. All other investigated CAM classes led to the formation of capsid structures with normal size and shape. In addition, immunofluorescence staining of HepAD38 cells showed that almost all CAMs led to a shift in the equilibrium of HBcAg localization towards the cytoplasm. However, cytoplasmic HBcAg in isolated aggregates were only observed in HAP-treated cells. In summary, we could show that HAPs seem to form a unique group among the CAMs, while all other CAMs have a common mode of action. It remains unclear, however, whether these differences in mode of action will have an impact on the goal to achieve HBV cure in the clinic.

17. IMB-Z Inhibits Enterovirus 71 Replication Through Regulating the Host Apolipoprotein B Messenger RNA-Editing Enzyme Catalytic Polypeptide-Like 3G

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Enterovirus 71 (EV71), as a single-stranded positive-sense RNA virus, is a common enterovirus that usually causes hand, foot and mouth disease (HFMD). Currently there is no effective antiviral drug in the clinic to treat EV71-induced HFMD. Host cellular factor apolipoprotein B messenger RNA (mRNA)-editing enzyme catalytic polypeptide-like 3G (A3G) is a cytidine deaminase that inhibits a group of viruses including human immunodeficiency virus-1 (HIV-1), hepatitis C virus (HCV), hepatitis B virus (HBV). In the continuation of our research on A3G, we found that introduction of external A3G inhibited EV71 replication and knockdown of endogenous A3G enhanced EV71 replication. Treatment of IMB-Z, a *N*-phenylbenzamide derivativeřinhibited EV71 replication in the infected cells through upregulating A3G expression. Furthermore, IMB-Z could increase the level of A3G incorporated into EV71 particles and reduce the infectivity of the progeny virus. However, G/A hypermutation in the EV71 genome were not detected and the cytidine deaminase activity of A3G was not required for an anti-EV71 effect by the C-terminal mutants (H257R, E259Q). Conclusion: A3G appears to be a cellular restrict factor against EV71 and IMB-Z could be a potential lead or supplement for the development of new anti-EV71 agents in the future.

18. Targeting RNA Methyltransferase METTL3 Reverses Drug Resistance in Influenza A Virus

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Infection with Influenza virus can cause severe respiratory illness and inflammation that can lead to organ failure and death. Neuraminidase inhibitors zanamivir and oseltamivir are effective therapies in treating influenza, however drug resistant strains are becoming increasingly common. Therefore, understanding the mechanism of drug resistance is critical for finding therapeutic approaches in treating drug-resistant influenza. In this study, we report acquired resistance with zanamivir in influenza A (IVA) virus leads to increased N6-adenosine methylation (m6A) of viral RNA, a modification previously demonstrated to alter RNA structure and function. m6A in IVA RNA is controlled by host methyltransferase METTL3 and demethylase FTO. We show treatment of IVA with 3-Deaza-adenosine (3DZA), a chemical inhibitor of m6A decreases resistance, whereas inhibition of m6A with meclofenamic acid (MA) increases resistance by altering viral RNA methylation. Using these findings, we designed oligonucleotide inhibitors that are steric blockers of METTL3 and demonstrated that treatment of host cells with inhibitory oligos significantly reduced m6A methylation in both host and viral RNA. Importantly, co-treatment of IVA with METTL3 inhibitor with zanamivir inhibited m6A levels in viral RNA and resulted in inhibition of drug resistant virus compared to control oligo treated group. In summary, our findings show, zanamivir resistant influenza viruses have increased m6A, and targeting host METTL3 enzyme with oligo nucleotide inhibitors in combination with zanamivir can effectively reduce viral m6A levels and reverse drug resistance.

19. Development and Validation of a High-Throughput Screen to Identify Macrocyclic Inhibitors Targeting the Zika Virus E Protein

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Zika virus (ZIKV) infected over 500,000 individuals in the recent epidemic in the Americas, leading to thousands of cases of congenital birth defects in children born to infected mothers. Currently, there are no approved countermeasures available to combat ZIKV infection. Most of the potential inhibitors of ZIKV target the NS5 RNA polymerase or the NS2B-NS3 protease. ZIKV E, which mediates both the attachment and fusion steps of viral entry, represents a novel target for direct-acting antivirals that inhibit the virus at the beginning of its replication cycle. We found that GNF-2, a small molecule inhibitor of the dengue virus E protein, also has modest activity against ZIKV. We utilized a biotinylated analog of GNF-2 and recombinant ZIKV E protein to establish a high-throughput competitive luminescence proximity assay and screened a commercial library of over 23,000 macrocyclic compounds for inhibitors of ZIKV E. We selected 47 primary screening "hits" and found that 43 of these compounds exhibit concentration-dependent activity in the primary assay. Additionally, 39 of these macrocycles have anti-ZIKV activity with $PRNT_{50}$ < 30 μM, with 6 possessing strong inhibition ($PRNT_{50}$ < 4 μM). The majority of the 47 macrocycles chosen as primary screening hits have limited cytotoxicity with CC_{50} > 100 μM, including many of the more potent inhibitors. Excitingly, many of these compounds also inhibit dengue virus, indicating that some of the identified macrocycles may have utility against multiple flavivirus pathogens.

20. Antiviral and Anti-Inflammatory Activity of Budesonide against Human Rhinovirus Infection Mediated Via Autophagy Activation

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Human rhinovirus (HRV) infection causes more than 80% of all common colds and is associated with severe complications in patients with asthma and chronic obstructive pulmonary disease. To identify antiviral drug against HRV infection, we screened 800 FDA-approved drugs and found budesonide as one of the possible drug candidates. Budesonide is a corticosteroid, which is commonly used to prevent exacerbation of asthma and symptoms of common cold. Budesonide specifically protects host cells from cytotoxicity following HRV infection, which depend on the expression of glucocorticoid receptor. Intranasal administration of budesonide lowered the pulmonary HRV load and the levels of IL-1ř cytokine leading to decreased lung inflammation. Budesonide regulates IL-1ř production following HRV infection independent of inflammasome activation. Instead, budesonide induces mitochondrial reactive oxygen species followed by activation of autophagy. Further, the inhibition of autophagy following chloroquine or bafilomycin A1 treatment reduced the anti-viral effect of budesonide against HRV, suggesting that the antiviral activity of budesonide was mediated via autophagy. The results suggest that budesonide represents a promising antiviral and anti-inflammatory drug candidate for the treatment of human rhinovirus infection.

22. Identification of Novel Drugs against Yellow Fever Using High Content Screening

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The Flavivirus is a large genus comprising different pathogenic arthropod-borne viruses with significant public health impact in different parts of the world and with potential of emerging in previously non-endemic regions. The Yellow fever and Dengue viruses are some of the most important human pathogenic flaviviruses, causing disease with similar symptoms that can evolve to severe disease. There is no specific antiviral available for the treatment of dengue or yellow fever. The objective of this study was to develop a high content screening assay, consisting on human hepatoma cell line infected with yellow fever or Dengue serotype 2 viruses, to screen a library of 1280 pharmacological active compounds. From 1280 compounds screened, 12 compounds were active against both viruses, highlighting the potential of finding compounds with broad antiviral activity in diversity libraries. The top 27 anti-YFV compounds were selected for further activity determination in dose-response and 5 compounds presented selective activity against YFV infection and only two of them, brequinar and U-73343, were also active against DENV. This assay allows us to screen a large amount of compounds in a short time span, speeding up the drug discovery and development of potential antiviral chemotherapies for these diseases.

23. 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.

The project envisaged that use of receptor-mediated endocytosis may permit the realization of potential of drug targeting that reduces 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.

24. High Content Screening/Imaging of Differentiated Human Primary Neural Cells Identifies Drug Candidates That Inhibit TBEV Infection.

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Tick-borne encephalitis virus (TBEV) is a leading cause of human neuroinfections in Europe and Northeast Asia. Despite its high medical relevance, no specific antiviral therapy is currently available. Development of more relevant *in vitro* infection models and discovery of new broad-spectrum antiviral agents are needed to address this unmet demand. We describe a cell-based, high-content approach using an *in vitro* model consisting of primary neural cells differentiated from human foetal neural progenitor cells (hNPCs) for discovery of anti-TBEV compounds. The mixed culture constituted of neurons, astrocytes and oligodendrocytes represent a more relevant and predictive cellular system for the identification of antiviral molecules actives against neurotropic viruses. We screened a library of 100 compounds for their ability to block TBEV infection in our model. We monitored the effect of compounds by quantification of infected cells stained with an antibody targeting the envelope protein of the virus, while simultaneously evaluating cytotoxicity. Established anti-flaviviral drugs and others that had no previously known antiviral activity were identified as inhibitors of TBEV infection. Several drugs previously identified on cell lines as anti-flaviviral candidates, were cytotoxic and did not reduce TBEV infection in our model. Thanks to the high content analyses, we identified and quantified mainly on DAPI staining, the neurons and astrocytes. We were able to identify which cells were targeted by TBEV and discriminate the action of drugs on these two cell populations. Our findings demonstrate that neural cells differentiated from hNPCs provide a cellular paradigm exploitable in the search for active anti-flaviviral molecules.

25. Development of First-In-Class Encephalitic Alphavirus Inhibitors with *In Vivo* Efficacy

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Of the more than 30 alphavirus pathogens known, about a third contributes to human disease, and currently there are no FDA approved treatments available for any of them. Geographically, alphaviruses are widely distributed RNA viruses that cause rash, arthritis, encephalitis, and death in humans. Due to the absence of effective therapeutic countermeasures for these pathogens, the increased incidence of their prevalence with global climate changes, and the ease with which the encephalitic alphaviruses have been weaponized as biological threats, we have focused on the identification and development of small molecule inhibitors of Venezuelan- Western- and Eastern Equine Encephalitis Viruses (V/W/EEEV, respectively). Our first disclosure involved ML336, a potent, nontoxic amidine resulting from a unique chemical rearrangement that inhibited an VEEV-induced cytopathic effect, dramatically reduced *in vitro* viral titer, and afforded good *in vivo* protection in mice. Through the development of new synthetic methods and medicinal chemistry effort, further optimization revealed structure-activity relationships that have been leveraged to deliver a non-amidine scaffold with improved physiochemical properties and has translated to 100% survival in a VEEV-based lethal mouse infection model. Assessment of these compounds show promise against WEEV and EEEV in cell culture, and the virus-centric mechanism of action has been further refined. Hence, next generation amidines and novel non-amidine inhibitors are currently being optimized in parallel for *in vivo* efficacy and further drug development milestones.

26. C2 and C7 Arylated Tryptophan Trimers and Tetramers As Dual HIV and Enterovirus 71 Entry Inhibitors

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We have recently reported a family of Trp dendrimers that are dual inhibitors of HIV and EV71.[1-3] The prototype compound of this family (AL-385) is a pentaerythritol derivative with 12 Trps on the periphery.[2] In the present study, we investigated whether truncation of the prototype could lead to reduced size compounds with retention of the activity. With this aim, analogues of the prototype, with only 3 or 4 Trps, were first prepared, resulting completely inactive against both viruses. To restore the activity, aromatic rings functionalized with different groups at the N1, C2 and C7 positions of the indol moiety were introduced.

Only compounds of general structure A and B, bearing phenyl (di)carboxylate groups at the C2 or C7 positions of the indole ring, were active against HIV and EV71, while the N1 arylated compounds were inactive against both viruses. The synthesis and antiviral activity against HIV and EV71 of these compounds will be presented.

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27. 2,4,6-Trisubstituted Pyrimidines As Potent HIV Non-Nucleoside Reverse Transcriptase Inhibitors

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As a continuation of our previous research,[1] novel 2,4,6-trisubstituted pyrimidines (Fig. 1), as potential non-nucleoside reverse transcriptase inhibitors (NNRTIs) derived from diarylpyrimidine (DAPY),[2] were prepared by new synthetic approach. The position 2 of pyrimidine ring was substituted by 4-cyanophenylamino arm in accordance with FDA (U. S. Food and Drug Administration) approved drugs etravirine and rilpivirine, the position 4 by *o,o*difluoroaniline, and the position 6 by a small substituent. Structure and anti-HIV activity relationship (SAR) study was performed on a series of some 20 compounds. The most potent derivative from the series, so far, exhibited low nanomolar anti-HIV-1 activity (EC50 = 4.4 nM) with no significant toxicity (CC50 > 57.1 μM).

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28. Protective Efficacy of a ZIKV VLP Vaccine in a Lethal Mouse Model

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The Zika virus (ZIKV) pandemic has resulted in significant morbidity and mortality around the world and there is a need for an effective vaccine to prevent disease. A candidate ZIKV virus-like particle (VLP) vaccine was tested in a lethal mouse model of ZIKV disease at doses of 10, 1, or 0.1 μg with and without an alum adjuvant and administered via intramuscular injection. Male and female AG129 mice were vaccinated with ZIKV VLP following a prime/boost schedule prior to challenge with a Puerto Rican isolate of ZIKV. Vaccination in the absence of adjuvant was dose-responsive, while the co-administration of vaccine and an alum adjuvant resulted in consistent production of neutralizing antibody titers, regardless of dose. Complete protection was observed in mice vaccinated with the top two doses of adjuvanted ZIKV VLP, with 90% protection at the lowest dose. Mice vaccinated with ZIKV VLP alone had a dose-responsive survival rate. Average weight change in all ZIKV VLP-vaccinated mice was similar to that of normal controls over the course of 3 weeks, while average weight of alum-vaccinated, infection controls steadily declined after infection. Viral RNA titer in day 5 serum was significantly reduced in a dose-responsive manner in all vaccinated mice, as compared with infected controls. This study demonstrated potent protective efficacy of a ZIKV VLP vaccine in a lethal mouse model. Further studies are planned to evaluate this vaccine in a non-human primate model prior to enrollment in clinical trials. [Supported by HHSN272201000039I from the Virology Branch, NIAID, NIH]

29. Progress and Resources of NIAID's Antiviral Development against Biodefense and Emerging Viral Diseases

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National Institute of Allergy and Infectious Diseases(NIAID) carries out the major mission of NIH in researching and developing medical countermeasures against biodefense and emerging viral diseases, also known as the NIH Select Agent Category A to C. Since the inception in 2005, the Drug Development Section within Office of Biodefense Research, Research Resources, and Translational Research is the integral component of NIAID's drug development effort by advancing promising preclinical antiviral programs into Phase 1 and 2 clinical evaluation. We work with other US government agencies, including HHS BARDA, FDA, and CDC, and DoD DTRA and DARPA, as well as other non-government organizations to respond to public crisis and unmet therapeutic needs. Over a decade, a number of antiviral programs against a spectrum of viral diseases such as smallpox, influenza, Dengue, Chikungunya, West Nile Fever, Ebola, Marburg, SARS, and MERS, have been supported by our group for lead optimization, clinical candidate selection, preclinical or clinical development. Some of these were tested for efficacy in the relevant animal models to fulfill the FDA's of Animal Rule for product licensure. Examples of successful programs under our support will be presented, and lessons shared. Also presented are available preclinical and clinical supports and resources from NIAID to the antiviral research community.

30. Targeting HIV-Infected Brain to Improve Stroke Outcome

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In the era of highly active antiretroviral therapy, the HIV prognostic has changed to a chronic disease. While the virus is repressed, several co-morbidities, including cardiovascular disease are still present. HIV positive individuals are more at risk of having strokes and also suffer from a less favorable recovery prognostic. Our hypothesis is that despite efficient HAART, residual HIV presence can contribute to stroke severity. In addition, we also hypothesize that efficient treatment using high CNS penetration effectiveness (CPE) drugs could benefit disease outcome by targeting CNS reservoirs. Our previous publications using the EcoHIV mouse model demonstrated that infection affects the blood-brain barrier. In the current study, we demonstrated that infection by EcoHIV significantly increased infract size when compared to mock infected animals and also reduced injury recovery. Upon further examination, we were able to demonstrate that stroke increased HIV presence in the affected hemisphere, with infected cells situated near the infract area. The majority of cells harboring the virus were from the macrophage/microglial lineage. We also detected a trend for an increase in inflammatory markers in EcoHIV infected stroke mice, especially those associated with the monocyte/macrophage/neutrophil response. We are currently investigating the potential therapeutic efficacy of targeting the HIV CNS reservoir using a high CNS penetrating efficacy therapy, and preliminary results indicates a significant benefit high CPE therapy. The successful implementation of this regiment would be highly beneficial in HIV patients at risk of cerebrovascular disease.

31. Chiral Substituted Pyrimidines As Potent HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors

Lucie Œchová, M.S.[1,2], Ondřej Baszczynski, Ph.D.[1], David Šaman, Ph.D.[1], George Stepan, Ph.D.[3], Eric Hu, Ph.D.[3], Eric Landson, Ph.D.[3], Petr Jansa, Ph.D.[3], Zlatko Janeba, Ph.D.[1], Petr Šimon, Ph.D.[1]
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Non-nucleoside reverse transcriptase inhibitors (NNRTIs), in combination with nucleoside reverse transcriptase and protease inhibitors, have become a cornerstone in acquired immune deficiency syndrome (AIDS) therapy. NNRTIs derived from diarylpyrimidine (DAPY family) represent a wide group of derivatives, which were subjected to numerous SAR studies.[1] Derivatives with a stereogenic center were investigated in cases where a separation of enantiomers lead to the improvement of anti-HIV activities.[2] Herein, we discuss a new synthetic approach and anti-HIV properties of novel substituted pyrimidines (Fig. 1). The pyrimidine ring was substituted with 4-cyanophenylamino moiety and with another aromatic system attached through various linkers Y.[3] Enantiomers of derivatives bearing a hydroxymethylene linker (Y = CHOH) were separated by chiral chromatography and their antiviral properties were studied.

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33. The Hepatitis C Virus RNA-Dependent RNA Polymerase Shuttles Incoming Nucleotides to the Active Site Through Successive Local Magnesium-Dependent Rearrangements

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RNA viruses synthesise new genomes in the infected host thanks to dedicated, virally-encoded RNA-templated RNA polymerases (RdRp). As such those enzymes are prime targets for antiviral therapy, as spectacularly exemplified recently in the case of hepatitis C virus (HCV). The HCV RdRp NS5B has become one of the best-characterised viral polymerases, both biochemically and structurally. Here we use molecular modelling and molecular dynamics simulations, starting from the available crystal structures of HCV NS5B in complex with template-primer duplexes, to address the question of ribonucleotide entry into the active site of viral RdRp. Tracing the possible passage of incoming UTP through the entry tunnel, we find that direct access to the active site is checked by successive mobile loops. A magnesium-bound nucleotide will bind next to the first loop and interactions first with the triphosphate moiety, then with ribose and base orient it base-first in the tunnel. Dynamics of active site residues then allow the nucleotide to interrogate the RNA template base prior to nucleotide insertion into the active site. These dynamics are finely regulated by a second magnesium dication, thus coordinating the entry of the correct magnesium-bound nucleotide with shuttling of the other magnesium necessary for the two-metal ion catalysis. This entry mechanism with limited, magnesium-dependent rearrangements along the path of the nucleotide is specific to viral RdRps and explains in part how 2'-modified nucleotides can be so successful as drugs against RNA viruses.

34. *In Vitro* Identification of a Src-Family Kinase Inhibitor As a New Therapeutic Option for Middle East Respiratory Syndrome Coronavirus Infection

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The Middle East respiratory syndrome coronavirus (MERS-CoV), first identified in Saudi Arabia, is an emerging zoonotic pathogen that causes severe acute respiratory illness in humans with a high fatality rate. Since its emergence, MERS-CoV continues to spread to countries outside of the Arabian Peninsula and causes sporadic human MERS infections with imported cases such as the 2015's outbreak in South Korea. Current therapeutic options of MERS-CoV are mainly adapted from previous reports of severe acute respiratory syndrome (SARS) therapies. In search of new potential candidates for treatment of MERS-CoV infection, we screened a library composed of 2,354 clinically approved drugs and pharmacologically active compounds and identified a set of compounds that inhibited *in vitro* MERS-CoV infection. Among these compounds, saracatinib, a Src-family kinase (SFK) inhibitor, was identified as a potent inhibitor of MERS-CoV replication. In addition, our results suggested that saracatinib potently inhibits MERS-CoV at early steps of viral RNA replication via a yet-unidentified target, which most probably is one or multiple proteins involved in the Src-family kinase signal pathway. The data presented in this study suggested a new therapeutic option for the control of MERS-CoV infection using an anticancer drug.

35. 2-Thiouracil Derivatives Downregulate Human Adenovirus Replication

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Human adenoviruses (HAdVs) are a group of viruses that can cause the infections of the respiratory tract, eyes, intestines, urinary tract, and nervous system. Currently, specific therapy for adenovirus infection, other than supportive and symptomatic treatment, is not available. Therefore, we propose to assess the anti-adenoviral activity of newly synthesized thiouracil derivatives.

To evaluate the inhibitory potency of these compounds HEK293 cells were infected with HAdV 5 at an MOI of 1 FFU/cell. The compounds at concentrations of 0.5, 2.5, 5, 10, 15 and 25 μM were added 3 h post infection. 24 h later newly synthesized viral genomes were detected via quantitative real-time PCR. Six of tested 2-thiouracils reduced adenoviral replication by more than 90% compared to control. Notably, we observed a strong reduction of HAdV-5 genome replication in adenovirus-infected HEK293 cells treated with Z556 (IC_{50} 0.06 μM) and Z557 (IC50 0.33 μM) as compared to DMSO treated cells. As positive control we used HEK293 cells treated with 2-{[2-(benzoylamino) benzoyl]amino}-benzoic acid (IC_{50} 6.9 μM). All compounds were non-toxic at effective concentrations according to MTT test. TC_{50}s of 405.7 μM and 172.7 μM were observed for Z556 and Z557, correspondingly, the 2-thiouracil derivatives with the highest level of antiviral activity.

We have shown that 2-thiouracil derivatives inhibit adenoviral replication in the low micromolar range and thus can be considered for the development of drug candidate to treat adenovirus infections. The work was supported by grant of the President of the Russian Federation for state support of young scientists (řř-1746.2017.7).

36. Computer-Aided Discovery and Characterization of Novel Ebola Virus Inhibitors

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The Ebola virus (EBOV) causes severe human infection that lacks effective treatment. A recent screen identified a series of compounds that block EBOV-like particle entry into human cells (Figure 1). Using data from this screen, Quantitative Structure-Activity Relationship (QSAR) models were built and employed for virtual screening of a ~17 million compound library. Experimental testing of 102 hits yielded 14 compounds with IC50 values under 10 μM, including several sub-micromolar inhibitors, and more than 10-fold selectivity against host cytotoxicity. These confirmed hits include FDA-approved drugs and clinical candidates with non-antiviral indications, as well as compounds with novel scaffolds and no previously known bioactivity. Five selected hits inhibited BSL-4 live-EBOV infection in a dose-dependent manner, including vindesine (0.34 μM). Additional studies of these novel anti-EBOV compounds revealed their mechanisms of action, including the inhibition of NPC1 protein, cathepsin B/L, and lysosomal function. Compounds identified in this study are among the most potent and well-characterized anti-EBOV inhibitors reported to date (Figure 2).

37. The Novel Nucleoside Analogue LJ-4269 Inhibits Coronavirus Replication.

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The emergence of the zoonotic coronaviruses (CoVs) causing severe acute respiratory syndrome (SARS) in 2003 and Middle East respiratory syndrome (MERS) in 2012 has highlighted their potential to cause significant human disease and constitute global pandemic threats. However, to date there is no effective antiviral therapy for the treatment of CoV infections in humans. The continuous development of new nucleoside analogues is an important general approach in antiviral drug development, but for CoVs this group of inhibitors has been explored to a very limited extent only.

Previously, during a cell culture-based screen of selenonucleoside and carbocyclic nucleoside analogues, compound LJ-4269 was identified as an inhibitor of the replication of both SARS- and MERS-CoV, with an EC_{50} in the low-micromolar range. Time-of-addition experiments indicated that MERS-CoV RNA synthesis as well as the production of progeny virus were most efficiently inhibited when cells were pre-treated with LJ-4269. Resistant MERS-CoV variants were now selected by passaging of the virus in the presence of increasing concentrations of the compound. Sequence analysis revealed mutations located in to the sequences encoding nsp3, nsp10, nsp13, nsp16, ORF5 and the M protein. Currently, using a MERS-CoV full-length cDNA clone, we are reverse engineering these mutations into the viral genome, in order to establish which mutations are associated with drug resistance. In addition, prodrugs and pyrimidine derivatives of LJ-4269 are being tested to elucidate the mechanism of action and evaluate which strategies can be used to further optimize the inhibitory activity of this analogue.

38. Structure-Activity Relationship Study of Itraconazole, a Broad-Range Inhibitor of Enterovirus Replication That Targets Oxysterol-Binding Protein

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Enteroviruses (eg. poliovirus, enterovirus, coxsackievirus, and rhinovirus) are a large group of human pathogens causing various mild and more severe diseases such as acute flaccid paralysis, hand-food-and-mouth disease, conjunctivitis, aseptic meningitis and common cold. Currently, only vaccines against poliovirus and enterovirus-A71 are available but no antiviral compound to treat enterovirus infections is on the market. One approach to develop broad-spectrum anti-enterovirus inhibitors is the development of host-targeted antivirals. Recently, we reported that the FDA-approved antifungal drug itraconazole has broad-spectrum antiviral activity against enteroviruses, cardioviruses (both belonging to *Picornaviridae*) and hepatitis C virus (belonging to *Flaviviridae*) by inhibiting oxysterol-binding protein (OSBP), a cellular lipid shuttling protein. In this structure activity relationship study, we analyzed the important chemical features of ITZ for inhibiting the function of OSBP and virus replication. Therefore, we used the cardiovirus encephalomyocarditis virus as a model. The backbone structure of itraconazole, consisting of five rings, and the *sec-*

butyl chain are important for OSBP inhibition and antiviral activity. In contrast, the triazole moiety, which is critical for antifungal activity, is not required for antiviral activity and OSBP inhibition. Importantly, we observed a good correlation between antiviral activity OSBP redistribution, confirming previous conclusion that ITZ exerts its antiviral activity through OSBP. Furthermore, we use *in silico* studies to explore how ITZ could bind to OSBP. This structure activity relationship study determined the important chemical features of ITZ towards virus inhibition and can contribute to novel ITZ-derived compounds that are specific towards OSBP.

39. Understanding Flavivirus Pathogenesis: Hijacking of Human Proteins by Non-Coding Viral RNA

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In an effort to unravel why flavivirus pathogens such as the dengue (DENV), yellow fever (YFV) and Zika virus (ZIKV) prove to be so harmful, this project focuses on the key virulence factor 'subgenomic flaviviral RNA' (sfRNA). These non-coding RNAs are shown to be highly conserved, essential for pathogenesis and a critical determinant of the epidemic potential of flaviviruses. SfRNAs mainly affect the host cell by binding specific RNA-binding proteins, disturbing a range of cellular pathways. Unravelling these interactions between viral non-coding RNAs and host proteins can lead to new molecular insights into flavivirus pathogenesis and potentially provide new and unique targets for antiviral intervention.

Using a unique yeast three-hybrid (Y3H) method, an human ORFeome library comprising more than 12.000 ORFs was screened for sfRNA-interaction and 73 putative sfRNA-interacting proteins were identified. Subsequently, an orthogonal yeast screen was performed, testing 25 of our top hits for interaction with a larger panel of flavivirus sfRNAs (DENV-2, YFV, Modoc, WNV, ZIKV strain MR766 and a Brazilian clinical ZIKV isolate) and 3 proteins showed broad-spectrum interaction with the full panel of flaviviruses. To confirm this sfRNA-protein interaction in a mammalian cell, an RNA-pulldown assay has been set up in a DENV-2 replicon cell line and preliminary data confirm the interaction between our first tested protein and the DENV-2 sfRNA.

40. Viral RNA Modification

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Viruses are a major force that shapes the evolution of all organisms. Nevertheless, there are surprisingly scarce data on RNA modifications or RNA conjugates in any type of viruses. Development of vitally important methods for the sensitive analysis of RNA modification enabling detailed studies of the chemical structure of various RNA entities (e.g. NAD captureSeq)[1] began only recently and they have not been applied to viral RNA. The simplicity of viral genomes and well-described structure and machinery make them a perfect model system for studying the role of new RNA conjugates. In our group, we combine various techniques such as LC/MS, next generation sequencing (NGS) or chemical biological methods for understanding the chemical structure of viral RNA. So far, we have focused on HIV-1 and some eukaryotic viruses from order of Picornavirales (examples of (+)ssRNA viruses). We analyzed RNA of pure viral particles as well as infected cells by LC/MS. The surprisingly high level of various methylations in viral RNA led us to application and development of specific profiling techniques in combination with NGS. We believe that our research will comprehensively provide new insight into the mechanism of action of various viral RNA in host cells.

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41. The *In Vitro* Characterisation of RV521, A Small Molecule Respiratory Syncytial Virus Fusion Inhibitor in Clinical Development

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RSV is a major cause of lower respiratory tract infections, manifesting as bronchiolitis or pneumonia in infants, children, and elderly and immunocompromised adults. No effective antiviral treatment currently exists.

RV521 is an oral small molecule RSV fusion inhibitor in development for the treatment of RSV infection in infants and adults. Therapeutic administration of RV521 safely and effectively reduced viral load and disease severity in a Phase 2a human RSV challenge model.

In *in vitro* plaque reduction and RSV F protein cell-cell fusion assays RV521 exhibits potent antiviral effects. To further characterise RV521 we performed spreading culture infections and demonstrate that RV521 is able to significantly reduce viral load when administered up to 32 hours post-infection.

RV521 has relatively low human plasma protein binding. We have performed RSV plaque and fusion assays in the presence of either human serum, or physiological concentrations of the human serum proteins ř1-Acid Glycoprotein (AAG) and human serum albumin (HSA) in the assay media. RV521 potency is not dramatically affected by the presence of these human serum components.

We have also investigated the antiviral effect of RV521 when administered in combination with RSV inhibitors that target a different mode of action. RV521 showed positive combination activity when combined with inhibitors of both RSV N- and L-proteins *in vitro* by plaque assay.

In summary, the *in vitro* characterisation of RV521 supports the further clinical evaluation of RV521 for RSV-infected adults and children.

42. The Natural Compound Silvestrol, a Selective Inhibitor of the RNA Helicase Eif4a, Has Potent Broad-Spectrum Antiviral Activity

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We have recently identified the plant compound silvestrol as a potent antiviral molecule to inhibit EBOV, CoV and Picornavirus replication (Biedenkopf et al., 2017, Müller et al., 2018). Silvestrol is a potent and selective inhibitor of the DEAD-box RNA helicase eIF4A. During translation initiation eIF4A is required to unwind stable RNA secondary structures in 5´UTRs of mRNAs to create a binding platform for the 43S preinitiation complex. Binding of silvestrol to eIF4A increases its affinity to the target mRNA, thereby stalling the helicase to its substrate. Interestingly, the 5´UTRs of several viruses contain stable RNA secondary structures. Therefore we asked, if viral protein synthesis is dependent on eIF4A to unwind viral RNA structures. Indeed, we have found that Silvestrol is active against several viruses with a (+)-strand RNA genome (e.g. ZIKV, HEV, CHIKV), (-)-strand RNA genome (EBOV), as well as against ASFV with a DNA genome. Moreover, silvestrol can inhibit viral protein synthesis when an eIF4A-dependent IRES element is used to initiate viral translation. Importantly, silvestrol shows remarkable low cytotoxicity in primary cells. We have established a dual luciferase reporter assay to analyze the antiviral silvestrol effects in more detail. We will present data on the antiviral broad-spectrum activity of silvestrol, on the RNA structure and sequence requirements that mediate silvestrol sensitivity in viral mRNAs and on the synthesis of silvestrol analoga. In the future we want to analyze the silvestrol effects in EBOV and CoV animal models.

43. Anti-Influenza Efficacy of Combined Use Aminocaproic Acid with the Benzodiazols' Derivatives *In Vitro*

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The influenza virus causes the greatest number of acute respiratory viral infections, which can lead to an exacerbation of chronic systemic diseases, to emergence of bacterial complications. So, the development of new anti-influenza drugs is an important task in medical science.

We have shown antiviral action of aminocaproic acid (ACA) and the benzodiazols' derivatives (C-26, C-29, C-60, C-66) *in vitro*. So, the combined use of ACA with the benzodiazols' derivatives was expected to be more effective.

The anti-influenza activity of the compounds was studied *in vitro* against strain A/Hong Kong/1/68 (H3N2) using tissue culture of chorio-allantoic membranes of 10-12-days chicken embryos (CAM).

Antiviral activity of the C-26, C-29, C-60, C-66 investigated in concentrations 10-3, 5ř10-4, 2,5ř10-4 mol. Concentrations ACA were 10-2, 5ř10-3, 7,5ř10-3 mol. Separately ACA or the benzodiazols' derivatives did not show an anti-influenza activity in these concentrations.

Potentiating of antiviral activity was observed when concentrations of C-26 were 10-3, 5ř10-4 mol and ACA was taken in all concentrations. The effect of potentiating act of C-29 with ACA was observed in all concentrations of C-29 and ACA. The effect of potentiating activity of C-60 with ACA was observed when concentrations of C-60 were 5ř10-4, 2,5ř10-4 mol and all concentrations of ACA. Potentiating effect of C-66 and ACA was observed when C-66 was at concentrations 10-3 mol and all concentrations of ACA.

Thus, combined application of compounds of different chemical nature allows to obtain a greater level of antiviral activity compared to the activity of each one.

44. Oligomerization Dynamics and Thermodynamics of Wt and Triple-Mutant Ebola VP35 Coiled-Coil Region From Self-Assembly Simulations

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The Ebola virus multi-functional virulence factor VP35 is a not-fully characterized putative therapeutic target. This protein consists of the dsRNA interacting C-terminal interferon inhibitory domain and of the N-terminal oligomerization domain.

Even if VP35 oligomerization has been recognized as an important step for its functions, up-to-now, the lack of structural information prevented to unveil the molecular details behind this process. In this work, the mechanism and the thermodynamics of VP35 oligomerization have been investigated by means of computational biology techniques focusing our attention on the protein coiled-coil region (F83-P120).

First, we predicted the structure of this region mediating self-assembly by means of non-standard homology-modeling techniques. Then, we combined coarse-grained and all-atom simulations to study oligomerization of the protein into dimers, trimers and tetramers. Finally, we rationalized the effect of the triple mutation (L90/93/107A) on the dimerization.

Our results show that the *wt* is able to self-assembly into coiled-coil dimers, trimers and tetramers. In contrast, the triple mutant shows poor propensity to even dimerize. Free-energy calculations highlight the key role of residues 90, 93 and 107 in stabilizing the coiled-coil dimer structure.

This *in silico* investigation, combined with in-house obtained experimental results (size-exclusion chromatography, native polyacrylamide gel electrophoresis, dsRNA binding biochemical assay and luciferase reporter gene assay), provided significant information on the VP35 multimer formation and stabilization, suggesting that higher order oligomeric states might originate as multiples of a fundamental dimeric unit.

45. Neplanocin A Derivatives As Selective Inhibitors of HBV Transcription

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Chronic hepatitis B virus (HBV) infection is currently treated with nucleoside analogs, such as lamivudine, entecavir, and tenofovir. Although these analogs are effective in HBV-infected patients, emergence of drug-resistant mutants during chemotherapy and viral reactivation after treatment interruption are major concerns in current antiviral agents against HBV. Therefore, it seems still mandatory to identify and develop novel inhibitors of HBV. We have reported in the previous ICAR that novel neplanocin A derivatives are selective inhibitors of HBV replication (AR-II-04-26: EC_{50}; 0.77, CC_{50}; > 100 μM, MK-III-02-03: EC50; 0.88, CC_{50}; 67.8 μM). They could also reduce HBsAg and HBeAg levels in culture supernatants of HepG2.2.15.7 cells and HBV-infected primary hepatocytes, but lamivudine and entecavir did not reduce the antigen levels. Furthermore, the cell-based cccDNA accumulation analysis revealed that the neplanosin A derivatives suppressed HBV RNA synthesis and HBsAg secretion. However, they did not affect HBV

Core, PreS1, PreS2/S, X promoter activity in HepG2 or HepG2.2.15.7 cells. These results suggest that the neplanocin A derivatives inhibit the expression of HBV RNA from cccDNA. Although their exact target molecule still remains unknown, AR-II-04-26 and MK-III-02-03 are considered as promising leads of novel anti-HBV nucleoside analogs with a different mechanism of action.

47. CRISPR/Cas9: A Molecular Tool to Selectively Target Merkel Cell Carcinoma.

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INTRODUCTION: Merkel cell carcinoma (MCC) is a highly aggressive skin cancer with a mortality rate of 33-46%. More than 80% of MCC cases are associated with integration of Merkel cell polyomavirus (MCPyV) and clonal expansion of transformed cells. MCPyV-positive (MCPyV$^+$) tumor cells express two viral oncoproteins: the small and large tumor antigens (sT and LT). The retinoblastoma sequestering activity of the LT confers oncogenic potential to MCPyV. Thus, we investigated the CRISPR/Cas9-mediated genome editing system as a new approach to target the viral oncogenes and impair tumor cells proliferation.

METHODS: We transfected MCPyV$^+$ cells with vectors expressing the Cas9 nuclease and a small guided RNA (sgRNA), specifically selected to direct the Cas9 nuclease activity to the genomic region of the viral tumor antigens. In addition, we evaluated the off target effects of the selected sgRNAs by transfecting HEK293T cells, a MCPyV$^-$ cell line.

RESULTS: The selected sgRNAs effectively induced insertion or deletion mutations (indels) in the genomic sequence of the viral tumor antigens. This caused a marked decrease of LT protein expression. Our CRISPR/Cas9 approach inhibited proliferation of the MCPyV$^+$ cells, while control HEK293T cells were unaffected. MCPyV$^+$ cells transfected with the sgRNAs targeting the viral tumor antigens presented both a slight increase in apoptosis and cell cycle deregulation.

CONCLUSIONS: Targeting MCPyV oncogenes with the CRISPR/Cas9 system is highly selective against MCPyV$^+$ cells, supporting its further validation as a potential approach for treatment of MCPyV$^+$ MCC lesions.

48. Posaconazole Inhibits Dengue Virus Replication by Targeting Oxysterol-Binding Protein

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Dengue virus (DENV) is associated with an estimated 390 million infections per year, occurring across approximately 100 countries in tropical and sub-tropical regions. To date, there are no antiviral drugs or specific therapies to treat DENV infection. Posaconazole and itraconazole are potent antifungal drugs that inhibit ergosterol biosynthesis in fungal cells, but also target a number of human proteins. Here, we show that itraconazole and posaconazole have antiviral activity against DENV. Posaconazole inhibited replication of multiple serotypes of DENV and the related flavivirus Zika virus, and reduced viral RNA replication, but not translation of the viral genome. We used a combination of knockdown and drug sensitization assays to define the molecular target of posaconazole that mediates its antiviral activity. We found that knockdown of oxysterol-binding protein (OSBP) inhibited DENV replication. Moreover, knockdown of OSBP, not other known targets of posaconazole, enhanced the inhibitory effect of posaconazole. Our findings imply OSBP as a potential target for the development of antiviral compounds against DENV.

49. The Host-Targeted Iminosugar UV-4B Inhibits Influenza Virus without Selecting for Resistance

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The development of antiviral drug resistance is a concern for many viruses, particularly for viruses with high mutation rates such as influenza. Antivirals that target host proteins required for viral replication are less likely to select for resistant viruses than drugs directly targeting viral proteins. The iminosugar UV-4B is a host-targeted glucomimetic that inhibits the endoplasmic reticulum ŕglucosidase I and II enzymes resulting in improper glycosylation and misfolding of multiple viral envelope glycoproteins. UV-4B has broad-spectrum antiviral activity *in vitro* against diverse RNA and DNA viruses. Oral treatment with UV-4B protects mice against lethal infection with mouse-adapted oseltamivir-sensitive and -resistant influenza A (H1N1 and H3N2) and B viruses. To examine the ability of influenza virus to generate

resistance against UV-4B, mouse-adapted influenza virus was passaged in mice in the presence or absence of UV-4B and virus isolated from lungs at the peak of virus replication was used to infect (~ 1 LD90) the next cohort of mice, for five passages. Deep sequencing was performed to identify changes in the viral genome during the passaging in the presence or absence of UV-4B. Only 7 nonsynonymous mutations were identified with an absence of apparent viral escape mutants following sustained exposure to UV-4B. Recombinant viruses containing individual or combinations of the nonsynonymous mutations unique to UV-4B pressure were still sensitive to UV-4B treatment in mice, providing additional evidence that there is a high genetic barrier to the generation and selection of escape mutants exposed to host-targeted iminosugar antivirals.

50. Identification of Obatoclax as Broad-Spectrum Antiviral Preventing Entry through Acidic Endosomes

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Many viruses enter cells through acidic endosomes, and thus this process constitutes a potential host cell target for antivirals. Chikungunya virus (CHIKV) is a mosquito-borne alphavirus, which is now found throughout the world in tropical and subtropical areas, causing in humans high fever, body rash, headache and severe joint pain that can persist for months or years. We discovered that obatoclax was strongly antiviral against CHIKV and another alphavirus, Semliki Forest virus (SFV), even when applied for very short periods during virus entry. We specifically established that obatoclax could neutralize the acidic environment of endosomes, thereby preventing virus entry. Thus, it was also active against other viruses requiring endosome acidification, including flaviviruses such as Zika virus, West Nile virus and yellow fever virus. It did not inhibit picornavirus species, which enter through acidification-independent pathways. Unexpectedly, the antiviral mechanism of obatoclax is entirely distinct from its previously established anticancer activities, which target the apoptosis-regulating Bcl-2 protein family, as shown by the fact that other Bcl-2 family inhibitors affected neither endosomal acidification nor virus entry. In further support of the proposed mechanism of action, partially obatoclax-resistant mutants of SFV had alterations in the viral fusion protein E1. We propose obatoclax as a potential broad-spectrum antiviral, as it generally inhibits virus entry through acidic endosomes at submicromolar concentrations.

51. HBV RNA Is an Early Predictor of Sustained HBeag Seroconversion in HBeAg-Positive Patients Treated with Pegylated Interferon Alpha

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BACKGROUND: As an important therapeutic drug against HBV infection, pegylated interferon ř (PegIFNř) offers a high rate of HBeAg seroconversion in a finite course of treatment, but biomarkers that accurately forecast interferon treatment response are yet to be elucidated. Here, we investigated whether HBV RNA may act as an early predictor to estimate HBeAg seroconversion during PegIFNř treatment.

METHODS: This study analyzed a cohort of 651 HBeAg-positive non-cirrhotic patients receiving a 48-week PegIFNř treatment and a 24-week follow-up. Serum levels of HBV RNA, HBV DNA, HBeAg, and HBsAg were measured at weeks 0, 12, 24, 48, and 72.

RESULTS: Compared to HBV DNA and HBsAg, HBV RNA levels dropped more significantly during the PegIFNř treatment from baseline to week 12. HBV RNA decline was associated with increased rates of sustained HBeAg seroconversion, while HBV RNA ≤ 1000 copies/mL at week 12 effectively predicted sustained HBeAg seroconversion. Multivariate analyses further revealed the significant predictive value of HBV RNA at week 12 and HBV DNA at week 24 but not at week 12 in response to interferon treatment success. Particularly, HBV RNA combined with HBV DNA, HBV genotype, and patient age significantly increased the prediction accuracy of sustained HBeAg seroconversion.

CONCLUSIONS: HBV RNA can serve as an early and effective predictor for estimating sustained HBeAg seroconversion in HBeAg-positive patients treated with PegIFNř. HBV RNA at week 12 shows its superiority in predicting sustained HBeAg seroconversion, highlighting its potential as an important marker in the early evaluation of treatment outcomes.

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52. A Rhinovirus Early Stage Inhibitor with a Novel Mechanism of Action

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In a cell-based screening effort, we identified a potent, broad-spectrum rhinovirus inhibitor with a novel mechanism of action. Similarly to capsid binders, compound 236580 (or destruxin B) exerts its activity during the early stages of the virus replication cycle, however, it does not protect RV14 from heat inactivation, Moreover, typical capsid binders-resistant (pleconaril and vapendavir) mutants do not show cross-resistance to destruxin B. These observations suggest a mechanism of action different than that of typical capsid binders. Destruxin B-resistant mutants acquired mutations at the N-terminus of VP1, which is known to be extruded during uncoating. This combined with the fact that destruxin B has been reported as a V-ATPases inhibitor, strongly points to an involvement of destruxin B in the inhibition of the V-ATPase-induced pH acidification of the entry endosomes, where viral uncoating occurs. Preliminary pH-dependent results indicate that destruxin B-resistant mutants are more stable in acidic pH as compared with RV14 wild-type. The resistant variants may hence bypass the pH-dependent uncoating mechanism (targeted by destruxin B), by employing a receptor-dependent uncoating like other acid-stable enteroviruses. More broadly, the N-terminus of the VP1 could represent a determinant of pH-sensitivity for enteroviruses.

53. Aniline-Based Inhibitors of Influenza H1N1 Virus Acting On Hemagglutinin-Mediated Fusion

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The influenza virus hemagglutinin (HA) is responsible for fusion between the viral and endosomal membranes during influenza virus entry. This fusion process can be blocked by compounds interfering with the acid-induced conformational change of HA.

After identifying two series of easily accessible anilines as inhibitors of influenza A/H1N1 virus, extensive chemical synthesis and analysis of the structure-activity relationship were performed. In Madin-Darby canine kidney cells infected with A/H1N1 viruses, the lead compound, 9d, displayed a 50% effective concentration of 1.5 to 5.5 μM and an antiviral selectivity index of 30. Inhibition of polykaryon formation in HA-expressing cells indicated that 9d and its analogue 14a interfere with low pH-induced membrane fusion mediated by the H1 and H5 (group 1) HA subtypes. Virus resistance as well as NMR experiments with the lead molecule 9d demonstrated that it interferes with HA-mediated fusion by binding to the HA stem and preventing its refolding at low pH. Molecular dynamics simulations suggest that ligand 9d is able to fill the "TBHQ pocket"[1] in the HAs of A/PR/8/34 and A/Virginia/ATCC3/2009. This implies that the "TBHQ pocket" represents a common and particularly relevant site for small-molecule HA fusion inhibitors, although distinct chemotypes are required to address the different polarity of this cavity in group-1 versus group-2 HA subtypes.

1) Russell et al., Proc. Natl. Acad. Sci. U. S. A. 2008, 105, 17736-17741.

54. Inhibition of Influenza and Human Corona Viruses by 1,4,4-Trisubstituted Piperidines

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The recent outbreaks of the highly pathogenic avian A/H5N1 and pandemic A/H1N1/2009 influenza viruses have emphasized the urgent need for the discovery of new anti-influenza compounds. On the other hand, Coronaviruses (CoV) are common etiological agents of acute respiratory tract infections and of potentially pandemic viruses (eg SARS-CoV or MERS-CoV), for which neither a vaccine nor an antiviral therapy is yet available, thus highlighting the need of agents with broad antiviral activity against multiple respiratory viruses.

The piperidine nucleus is an attractive drug scaffold present 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 broad spectrum antiviral compounds, several molecules of our diverse in-house library were screened against a panel of different influenza virus strains and human 229E coronavirus. Following this approach, we identified 1,4,4-trisubstituted piperidines as interesting hit compounds that display antiviral activity against influenza A/PR/8/34 virus (A/H1N1) and coronavirus (229E) in the low micromolar range. To investigate the structure-activity relationships, several analogues were easily synthesized, by a one-step Ugi four-component reaction, starting from commercially available amines, isocyanides, N-substituted piperidones and a variety of amino acids as carboxylic acid components. Mechanistic influenza experiments consisting of time-of-addition, resistance selection and functional assays for HA-mediated binding or membrane fusion with the most active compound will be reported.

55. Discovery of Broad-Spectrum Influenza Antivirals by Targeting Pro-Viral Host Factors

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Influenza viruses are responsible for annual epidemics and occasional pandemics, posing a great burden of disease worldwide.Both influenza A and B viruses infect human.Currently the mainstay of countermeasures against influenza virus infection is neuraminidase inhibitors. However, the only oral drug oseltamivir has a limited therapeutic window and has to be taken within 24-48 h after the onset of the symptoms. Moreover, the number of oseltamivir-resistant influenza viruses keeps increasing. Therefore there is a clear need for the next-generation of influenza antivirals. In pursuing broad-spectrum influenza antivirals with a high genetic barrier to drug resistance, we have discovered several host-targeting antivirals (HTAs) that inhibit multiple human clinic isolates of influenza A and B viruses in different cell lines. These HTAs include kinase inhibitors, molecular chaperon inhibitors, agonists/antagonists of cell receptors. Although a common concern regarding HTAs is the cellular cytotoxicity, we have found two strategies that could increase the selectivity of HTAs: 1) exploring allosteric kinase inhibitors. Although several kinase inhibitors have been shown to have broad-spectrum influenza antiviral activity, most of the reported antiviral kinase inhibitors have low selectivity as they are ATP-competitive inhibitors and often have off targets effects. In our study, we found that allosteric kinase inhibitors generally have a higher selectivity index (SI) than active site ATP-competitive kinase inhibitors because of their high target specificity. 2) Targeting host factors that are only activated upon viral infection. In this case, the therapeutic dose needed to suppress viral infection will not impair normal cellular function of the host factor.

56. Verdinexor, a Novel Inhibitor of Exportin-1, Shows *In Vitro* Efficacy against Opportunistic Viral Infections in the Immunocompromised Patient

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Recent advances in antiretroviral treatment have greatly prolonged the lifespan of immunocompromised individuals with HIV. Despite this, high rates of infections from opportunistic dsDNA viruses remain, and even slight changes to the immune landscape can lead to reactivation of latent viruses primarily associated with malignancies. Exportin-1 (XPO1) is a member of the karyopherin family of protein chaperones that serve to actively transport over 200 cargoes through the nuclear pore complex into the cytoplasm. Verdinexor is a novel selective inhibitor of nuclear export (SINE) compound that binds and inhibits XPO1. The result of verdinexor treatment is sequestration of critical host and viral proteins to the nucleus, where they are often unable to participate in viral replication. Additionally, verdinexor promotes an anti-inflammatory state that alleviates symptoms of infection and immunopathology. Verdinexor is IND-ready, orally bioavailable, and has performed well in *in vitro* animal models. Importantly, verdinexor targets a host protein, reducing the development of resistant viral mutants. Here, we present results of an *in vitro* screen of verdinexor performed as part of an NIH NIAID antiviral screening contract against KSHV, EBV, 2 strains of HPV, HCMV,

AdV, JCV, and BKV. Treatment with verdinexor was efficacious in most cases, with a range of EC50 values from 30nM (AdV) to 8.3μM (HPV-18) and selectivity index (SI) ranging from 3-30. Studies showing the reliance of these opportunistic viruses on intact XPO1 function, along with previous efficacy data for HIV, provide strong mechanistic rationale for the further development of verdinexor as an antiviral for immunocompromised patients.

57. A New Locus of Letermovir Resistance in the Human Cytomegalovirus UL56 Gene Revealed by *In Vitro* Exposure to Letermovir and Ganciclovir

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Letermovir is a human cytomegalovirus (CMV) terminase inhibitor recently approved as prophylaxis in stem cell transplant recipients. In further studies of emerging drug resistance, a baseline laboratory CMV strain was serially propagated in cell culture under a combination of letermovir and ganciclovir. In 8 experiments, UL56 gene mutations were detected beginning at 10 passages, including novel amino acid substitutions V236A, L328V and A365S, located in a codon range 229-369 previously associated with letermovir resistance. Outside this region, UL56 substitution C25F was detected at moderate letermovir concentrations in 2 experiments, as either the first detected mutation, or adding to a preexisting substitution V231L. In all cases, mutation at UL56 codon 325 conferring absolute letermovir resistance developed initially or eventually, including one instance of C325W as observed in a letermovir clinical trial. No UL97 kinase or UL54 DNA polymerase mutations relevant to ganciclovir resistance were detected until many passages after the first detection of UL56 mutations, including UL97 A591V and UL54 N408K, F412C, D515Y and A543P, all at >30 passages. UL56 substitutions V236A, L328V and A365S were shown to confer borderline or low-grade letermovir resistance, while C25F conferred 5.4-fold increased letermovir resistance (EC50) by itself and 47-fold in combination with V231L. The C25F mutant was not appreciably growth-attenuated. Evolution of resistance mutations sooner for letermovir than for ganciclovir is consistent with prior *in vitro* observations, and UL56 codon 25 is a genetic locus for letermovir resistance distinct from those previously described.

58. Deubiquitinating and Inhibition of Porcine Epidemic Diarrhea Virus Papain-like Protease 2

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First discovered in 1970s, porcine epidemic diarrhea virus (PEDV) is a coronavirus which infect the intestinal tract of pigs. PEDV can cause extreme diarrhea and dehydration and thus devastating for neonatal piglets. In Asia, live attenuated vaccine has been a major approach to control PEDV infection in the recent years. However, a new strain of virus, which is resistant to vaccination, arose in 2013 in USA and quickly spread all over the world. The considerable economic lost caused by the new strain raises the importance of further understanding of PEDV, as well as its inhibition strategy. In this study, recombinant PEDV papain-like protease 2 ($PL2^{pro}$) was produced and its deubiquitinating and proteolytic activity were delineated. Furthermore, several known papain-like protease inhibitors, such as 6-mercaptopurine, 6-thioguanine, disulfiram, and mycophenolic acid, were used to clarify their inhibitory effect against PEDV $PL2^{pro}$. Besides, the structure of $PL2^{pro}$ catalytic triad mutant, C100S, in complex with ubiquitin was solved by x-ray crystallography. The information provides accurate atomic model for better understanding PEDV $PL2^{pro}$'s inhibition mechanism.

59. 3′-Halo-5′-Norcarbocyclic Nucleoside Phosphonates as Potent Anti-HIV Agents

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More than three decades after the discovery of the Human Immunodeficiency Virus (HIV) as the etiologic agent of AIDS, there is no vaccine available for the prevention of AIDS and drugs are the only arsenal to treat HIV infections. However, current treatments do not allow the eradication of the virus but contain its replication at undetectable level with the obligation for the infected individuals to stay on treatment for life. This is a crucial issue because all existing anti-HIV drugs have long-term side effects and may be associated with the rapid emergence of resistant viral strains in case of faulty observance to treatment or suboptimal treatment. These concerns still promote the research for novel molecular-based anti-HIV drugs. Among these later, nucleoside and nucleotide analogues are an important class of anti-HIV drugs as illustrated with the clinical use of Abacavir, a carbocyclic nucleoside analogue, and Tenofovir disoproxil fumarate (TDF), the corresponding carbonate prodrug of (*R*)-9-(2-phosphonylmethoxypropyl)adenine (*R*-PMPA, Tenofovir), an acyclonucleoside phosphonate (Figure 1). As a part of our research on 5′-norcarbocyclic nucleoside phosphonates as potential anti-viral agents, we describe here the synthesis and the antiviral evaluation of their 3′-halo (iodo and fluoro) corresponding counterparts bearing purine bases.

60. Antiviral Activity of a Series of Indole Alkaloids against Emerging Flaviviruses

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During the past decades, mosquito-borne flavivirus infections have become a major public health problem. In addition, climate changes and a massive increase in international traffic have established these viruses as a global danger. Despite their impact on health systems and economies, no antiviral drugs are currently approved for the treatment of these infections. In this study, a promising class of compounds (indole alkaloids) was identified displaying a selective antiviral activity against yellow fever virus (YFV), as well as the four serotypes of dengue virus (DENV type 1-4) and this in the low micromolar range. Interestingly, the compounds were not active against Zika virus replication. Moreover, the antiviral activity was evaluated in several cell lines, including Vero cells (kidney monkey epithelial), Huh cells (hepatocarcinoma), A549 cells (lung adenocarcinoma) and insect C6/36 cells. Several *in vitro* tests were performed to pinpoint their mechanism of action. In order to define at which stage of the viral infection cycle the compounds are active a time-of-drug addition assay was performed. Reference compounds revealed that this class of compounds interferes with the virus replication stage. The results were confirmed by the use of the subgenomic replicon (containing only the non-structural proteins). Based on this assay, a clear reduction in the luciferase activity indicates the interference of the compounds with the viral non-structural proteins or the replication complex. Additional studies are in progress to identify the molecular target of these compounds and provide more insights in the main mechanisms flaviviruses use for infection and replication in humans.

61. Identification of a Druggable VP1-VP3 Interpromoter Binding Pocket in the Capsid of Enteroviruses.

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Coxsackievirus B (CVB) are human enteroviruses, responsible for a wide array of diseases, for which no prophylactic and therapeutic treatment is currently available. Capsid binders, the best-studied class of enterovirus inhibitors, bind in a VP1-pocket under the floor of the canyon and block virus attachment/uncoating. Capsid binders showed poor efficacy *in vivo* and selected also rapidly for drug-resistant variants. We here describe a novel benzene sulfonamide derivative (i.e. compound 17) as a selective inhibitor of CVB3 replication. Time-of-drug-addition and thermostability studies indicated that compound 17 interferes with an early step in the virus replication cycle. The compound is also not cross-resistant with known capsid binders and has a different mechanism of action than these molecules. Compound 17-resistant CVB3 variants carried mutations F76C, E78G, A98V and D133G in the VP1 protein, hitherto an unknown region for binding of inhibitors, which was confirmed by reverse genetics. Cryo-electron microscopy and image reconstruction followed by molecular docking of CVB3 with compound 17 unveiled a novel binding pocket formed by viral proteins VP1 and VP3 at the interface of two protomers around the 5-fold axis, comprised of the residues 75-78, 98, 155 of VP1 and the residues 233, 235-236 of VP3. Mechanistic studies revealed clearly that compound 17 does not block the binding to CAR receptor, but that it may interfere with DAF-binding. In summary, we identified a capsid binder with an entirely novel mechanism of action and a novel druggable pocket in the picornavirus capsid.

62. Novel PI4KB Inhibitor- Potent and Broadspectrum Antivirals against Enterovirus

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A rational design approach was used for development of novel PI4K inhibitors as potent broadspectrum antivirals against enterovirus.

Enterovirus are a serious health problem throughout the world. The enterovirus group includes hundreds of different viruses and the symptoms of infections can vary greatly. It is common that the symptoms are very mild or infection proceeds without noticeable symptoms but enterovirus can also cause serious diseases such as myocarditis septicemia, enchephalititis etc. and could be lethal. Despite intensive research, there are currently no approved drugs for enterovirus infections.

We will present the synthesis novel PI4KB inhibitors that is consider as host-targeting antivirals. Several scaffolds based on the same framwork have been produced and the structure-activity relationship of the compounds from a CPE based assay will be presented. Additional ADME/PK data will also be presented.

63. Verdinexor (KPT-335) Demonstrates Antiviral Activity against Multiple Emerging Influenza Strains

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Emerging recombinant influenza A (IAV) is a major public health burden. H1N1 has caused pandemic outbreaks and treatments are limited in scope as resistant viruses emerge. As such there is an unmet need for anti-influenza therapies. Verdinexor, an orally-bioavailable inhibitor of the nuclear export protein, exportin-1 (XPO1), reduces viral replication by inhibiting the nuclear export of influenza ribonucleoproteins (vRNP). Additionally, since XPO1 is a host protein critical for host cellular functions,genetic conservation is critical.

Verdinexor was tested against human and animal isolates of IAV using plaque reduction and growth curves for swine (H1N1), duck (H4N6, H5N3) and equine (H3N8) viruses and a human isolate (H3N2). To determine potential resistance, nucleoprotein (NP)-mutant virus susceptibility was tested by plaque reduction assays, dose-response inhibition and analysis of NP intracellular localization. Resistant viruses were rare, but were generated by repeated passage of virus in presense of sub-inhibitory concentrations of verdinexor. Fitness of drug resistant IAV was determined inferior to WT.

Verdinexor completely inhibited plaque formation at 1mM with a subsequent EC_{50} value of 0.18mM. Verdinexor displayed dose-dependent inhibition of virus replication with EC_{50} values ranging between 4-30nM with selective indicies of 87-650. Mutations in virus NP lead to resistance, however the mutant viruses were attenuated and susceptible to human MxA. These results suggest verdinexor is efficacious against diverse strains of IAV. While drug resistance is possible, we were unable to develop a strain with increased fitness, and thus, verdinexor may provide a novel and efficacious therapy for emergent influenza infections.

64. The Viral Polymerase Inhibitor 7DMA Reduces Zika Virus Replication in Reproductive Organs of Male Mice

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Zika virus (ZIKV) is an arthropod-borne virus that belongs to the family of the Flaviviridae. It is mainly transmitted through the bite of female Aedes mosquitoes. In 2008, the first case of sexual transmission of ZIKV was documented. This alternative route for transmission has gained more attention during the latest outbreak in the Americas. Although not contributing significantly to the transmission of ZIKV in areas where the Aedes mosquitoes are abundantly present, it may contribute to a large part of locally acquired cases in areas where the mosquitoes are absent. We previously established a robust animal model of ZIKV infection in AG129 mice. Interestingly, infection with ZIKV MR766 resulted in high levels of viral RNA in the testis of infected male mice. This was recently corroborated by others who have detected infectious virus in the reproductive organs of infected mice as early as day 3 pi. We earlier showed that the nucleoside analog 7-deaza-2'-C-methyladenosine (7DMA) elicits good efficacy against ZIKV infection in mice, hence our model is very suitable for the assessment of the *in vivo* antiviral efficiency of novel ZIKV inhibitors. We now show that 7DMA significantly lowers viral RNA and infectious virus titers in the testis and epididymis of mice infected with a Suriname isolate (SL1602), both at day 3 and day 7 pi. We thus propose that novel ZIKV inhibitors that are being developed, and to which we actively contribute, should preferably elicit activity in this model.

65. An Enterovirus Infection Model of the Murine Upper Respiratory Tract to Assess the Activity of Antivirals

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Rhinoviruses (RVs, genus *Enterovirus*) are mostly associated with mild, upper respiratory tract infections (RTI), but can also cause lower RTI which can lead to severe exacerbations of asthma and chronic obstructive pulmonary disease. Despite extensive efforts, no antiviral therapy is currently approved for the treatment of RV infections. This is in part due to the exclusive human tropism of RVs which hampers the development of relevant small animal models. To establish a respiratory infection model, we employed the use of an other enterovirus as a surrogate for RV, coxsackievirus B4 (CV-B4), which is a human enterovirus known to replicate efficiently in mice. The model development would allow the *in vivo* assessment of antiviral effects of newly identified compounds in physiological relevant tissue. The SCID mice inoculated intranasally with CV-B4 did not develop any symptoms of respiratory disease, but viral titers in the nasal

mucosa increased 60-fold on average until two days post infection (dpi) after which a plateau was reached, and this remained stable until four dpi. Treatment with a potent antiviral compound, which has similar *in vitro* activity against RV and CV-B4, resulted in a complete clearance of the virus, even when treatment was initiated 48h pi. In conclusion, we established a robust upper RTI model with significant physiological relevance to assess the efficacy of rhino/enterovirus inhibitors. Optimal treatment with a potent inhibitor completely cleared the virus from the nasal mucosa, even after a delayed start of treatment.

66. Genetic Variants Analysis of BK Polyomavirus Genome and of the Cellular *CMPK1* Gene from Kidney Transplant Recipients

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INTRODUCTION: BK polyomavirus (BKPyV) was discovered in the early 70s in a kidney transplant patient. The immunosuppression state of the patient can trigger reactivation of the virus, leading in 30% of the cases to graft loss. Currently, no specific antiviral treatment of BKPyV-nephropathy has been established. Immunosuppressive drug minimization, and cidofovir permit in some cases to control the viral replication and maintain graft integrity. However, in some patients, this therapeutic strategy does not allow the management of BKPyV infection and graft loss may occur.

METHODS: We genotyped BKPyV strains isolated from clinical samples (blood, urine, and kidney biopsy), obtained from renal transplant recipients (n=16) with BKPyV reactivation, using next-generation sequencing. In parallel, genetic variations in the cidofovir activation enzyme, i.e. UMP-CMP kinase (*CMPK1*), were assessed using Sanger sequencing of reverse-transcripted RNA isolated from kidney biopsies.

RESULTS: A series of genetic polymorphisms was identified in the large tumor (LT) antigen, specifically in patients who did not respond to the cidofovir-based treatment. In addition, we discovered concomitant presence of different subtypes of BKPyV in the blood and the urine of a patient. Several polymorphisms in the *CMPK1* gene were also identified resulting into amino acid changes (R42G, Q48H and N83S).

CONCLUSIONS: The presence of polymorphisms, both in the viral genome and in the *CMPK1* gene, may be responsible for variation in the treatment outcome of patients that reactivate BKPyV.

67. Analysis of Cidofovir (CDV) as an Anti-Tumor Drug and Evaluation of the Mechanisms Underlying CDV Resistance by Comparing HPV16+ Cervical Carcinoma (Siha), Siha CDV-Resistant ($Siha_{cdv}$) and Normal (Primary Human Keratinocytes - Phks) Cells.

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INTRODUCTION: Cidofovir is mainly known for its activity against DNA viruses although the drug has also antiproliferative effects. Cidofovir was demonstrated to inhibit the proliferation of viral (e.g. HPV16+ SiHa cervical carcinoma cells) and non-viral associated tumor cells. Our aim was to investigate differences between SiHa and $SiHa_{CDV}$ cells compared to PHKs to provide insights into cidofovir antitumor activity.

METHODS: Cell cycle-related proteins were analyzed by Western blot and DNA damage response (DDR) examined by accumulation of Rad51 foci and g-H2AX expression. CDV cytostatic effects and doubling time were investigated after knockdown of ATM, Chk1 or Chk2 (coordinators of DDR and cell cycle checkpoint response).

RESULTS: PHKs and SiHa cells accumulated in S-phase after 3 and 5 days (d) of CDV treatment while a slight accumulation in S-phase was observed for $SiHa_{CDV}$ and only SiHa cells underwent apoptosis (5d). CDV induced DNA damage and DDR activation as observed by increased phosphorylation (p) of ATM, Chk1/2, p53 and by decreased p-MDM2 in all cell types. S-phase arrest was related to increased p-pRb (S-phase transition) and decreased CDK2 expression (G2-phase transition) in SiHa and $SiHa_{CDV}$. Rad51 foci formation was induced in all cell types but foci only accumulated in SiHa cells. Similarly, g-H2AX (double-strand breaks marker) expression solely increased in SiHa cells (5d). ATM inhibition partially reverted $SiHa_{CDV}$ resistance while Chk1/2 inhibition induced CDV resistance in PHKs.

CONCLUSIONS: Expression of G1/S- and apoptosis-related proteins and the ability to repair DNA damage might be the main differences between PHKs, SiHa and $SiHa_{CDV}$.

68. Nitazoxanide Inhibits Human Norovirus Replication and Synergizes with Ribavirin by Activation of Cellular Antiviral Response

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BACKGROUND: Norovirus is the main cause of viral gastroenteritis worldwide. Even though norovirus gastroenteritis is self-limiting in immunocompetent individuals, chronic infection with debilitating and life-threatening complications may occur in immunocompromised patients. Nitazoxanide (NTZ) has been empirically used in the clinic and demonstrated effectiveness against norovirus gastroenteritis.

OBJECTIVES AND METHODS: In this study we aimed at uncovering the *bone fide* antiviral effects and mechanism of actions of NTZ and its active metabolite, tizoxanide (TIZ) against human norovirus (HuNV) using a stable subgenomic replicon model.

RESULTS: NTZ and TIZ, collectively referred to as thiazolide (TZD), displayed potent inhibition of HuNV replication with mean IC_{50} of 1.013 and 1.110 μg/mL, respectively. Mechanistic studies revealed that TZD activated cellular antiviral response and stimulated the expression of a subset of interferon-stimulated genes (ISGs), in particular IRF-1. Overexpression of exogenous IRF-1 inhibited HuNV replication; whereas IRF-1 knockdown largely attenuated the antiviral activity of TZD, suggesting that IRF-1 mediated TZD inhibition of HuNV. By using a JAK inhibitor CP-690550 and STAT1 knockout approach, we found that TZD induced antiviral response independent of the classical JAK-STAT pathway. Furthermore, TZD and ribavirin synergize to inhibit HuNV replication and completely deplete the replicons from host cells after long-term treatment.

CONCLUSIONS: In summary, our results demonstrated that TZD combated HuNV replication though activation of cellular antiviral response, in particular inducing a prominent antiviral effector IRF-1. NTZ monotherapy or combination with ribavirin represents promising options for treating norovirus gastroenteritis, especially in immunocompromised patients.

69. A Systematic Analysis of the Potential and Limitations of Epigenetic Inhibitors As Antiviral Drugs

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Many epigenetic inhibitors have been tested for their effects on viral replication, as potential tools in antiviral therapy. Epigenetic inhibitors would only be specific antivirals if viral and cellular chromatin are differently regulated. Surprisingly, most studies have focused on inhibitors targeting epigenetic modifications proposed to play similar roles in viral and cellular gene expression. The tested epigenetic inhibitors therefore dysregulate cellular gene expression, which may well mediate any antiviral effects.

We undertook a systematic approach to evaluate the antiviral potential of epigenetic inhibitors, testing 37 well characterized compounds against DNA (herpes simplex 1 [HSV], adenovirus 5 [Ad5] and vaccinia virus (VV)) or RNA (influenza A [IAV], vesicular stomatitis [VSV] virus) viruses with nuclear (HSV, Ad5, IAV) or cytoplasmic (VV, VSV) replication. HSV, VV and VSV were tested in Vero cells (for direct comparisons), HSV also in human primary fibroblasts, Ad5 in HEK293 and IAV in MDCK cells. Only four epigenetic inhibitors had no effects on viral replication. Twenty-four inhibited the replication of HSV and Ad5, but not that of VV, IAV or VSV, and two inhibited that of all viruses. However, only two epigenetic inhibitors achieved pharmacological levels of inhibition and seven activated HSV replication. Four of six histone acetylase inhibitors affected equally HSV replication and histone acetylation, whereas two inhibited HSV replication more than histone acetylation. Most epigenetic inhibitors affect nuclear replicating DNA viruses but not a cytoplasmic DNA virus or cytoplasmic or nuclear RNA viruses, indicating that they exert their antiviral activities mostly by modulating viral epigenetics.

70. 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 conventional strategies. Frequent booster dosing can be avoided by development of mucosal/adjuvant vaccine delivery system, which can produce bothhumoral and cell-mediated responses. The work envisaged usescombined hydrophilic(gelatin nanoparticles,GN) with a hydrophobic polymeric system(PLGA microspheres) which creates a biodegradable system for HBsAgdelivery.

GN& PLGA microspheres were prepared by double emulsification method and composite system by phase separation method.Composites were optimized and characterized *in-vitro* for their shape, size by Scanning &Transmission Electron Microscopy,%antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake. *In-vivo* study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. 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 receiving booster dose. Further, composite system generated effective sIgA antibody which was not elicited by marketed formulation. Thus, it could be concluded from 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 Hepatitisbut against all those diseases that invade host by mucosal surfaces.

71. Antibody Coated Liposomes for Transmucosal Vaccination

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The critical role of vaccine delivery system in "rational vaccine design" has been widely recognized. 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 sustaining release of HBsAg to evoke 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 in 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.

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. The strategy can be made more appropriate by determination of paracellular transport, nasal mucociliary clearance, mucosal toxicity assessment etc.

72. Galidesivir (BCX4430) Limits Rift Valley Fever Virus Infection and Disease Modeled in Syrian Golden Hamsters

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Rift Valley fever virus (RVFV) is a mosquito-borne pathogen endemic to sub-Saharan Africa and the Arabian Peninsula. Exposure to the virus can result in a spectrum of conditions ranging from self-limiting acute febrile illness to fatal hemorrhagic fever, encephalitis or retinitis that can result in varying degrees of blindness. There are no approved antiviral therapies or vaccines to treat or prevent severe disease associated with RVFV infection in humans. The adenosine analogue, galidesivir (BCX4430), is a broad-spectrum antiviral drug candidate with *in vitro* antiviral potency (EC50 of less than 50 μM) in more than 20 different viruses across eight different virus families. Here we report on the anti-RVFV activity of galidesivir in cell culture and in the hamster model of peracute RVFV infection by multiple drug administration routes. Intramuscular and intraperitoneal treatments effectively limited systemic RVFV infection as demonstrated by significantly improved survival outcomes and the absence of infectious virus in the spleen and the majority of the serum, brain, and liver samples collected from infected animals. Pharmacokinetic analysis of galidesivir showed high levels of drug in the plasma, with a rapid initial distribution phase consistent with rapid uptake into cells, and slow terminal elimination. Our findings support the further development of galidesivir as an antiviral therapy for use in treating severe RVFV infection, and possibly other related phleboviral diseases. *This work was supported by the National Institutes of Health (HHSN272201000039I and HHSN272201100019I).*

73. Broad Spectrum Virucidal Non Toxic Strategies

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Viral infections kill millions yearly. Most antiviral drugs are virus-specific and we lack treatment for the majority of viruses, moreover in the recent epidemics of Ebola and Zika, despite the huge effort, we failed in developing a specific antiviral in time. An ideal strategy to fight viral infections is to develop broad-spectrum antivirals that irreversibly inhibit viral infectivity. We designed sulfonic acid decorated gold nanoparticles that mimic cell attachment-receptors widely used by viruses (heparan sulfate proteoglycans). Viruses binding to these materials undergo an irreversible loss in viral infectivity with a structural deformation due to the multivalent and structural characteristic of the ligands. Virucidal assays, electron microscopy images and molecular dynamics simulations support the proposed mechanism. These nanoparticles show no cytotoxicity, and *in vitro* nanomolar irreversible activity against herpes simplex virus, human papilloma virus, respiratory syncytial virus, dengue and zika virus. Their activity is maintained *in vitro*, *ex vivo* in human cervicovaginal histocultures infected by HSV-2, and *in vivo* in mice infected with RSV. We then substituted the gold with a biocompatible sugar core, proving similar efficacy *in vitro*, *ex vivo* and *in vivo*. Finally, we are now developing the same multivalent strategy to target viruses dependent on sialic acid receptors, maintaining the virucidal activity *in vitro* and *ex vivo* and proving the potential applicability of this approach to all viral receptors.

74. Impact of Hiv-1 Subtype and Korean Red Ginseng On Aids Progression: Comparison of Subtype B and Subtype D

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The CD4+ T cell decline is about 4-fold more rapid in HIV-1 subtype D-infected patients (D patients) than in subtype B-infected patients (B patients). However, little is known about disease progression for subtype D in Asia owing to its low prevalence. We analyzed the decline in CD4+ T cells over 97 ± 59 months in 164 patients with subtype D and B infections based on the nef gene. The annual decline (AD) in CD4+ T cell counts was significantly greater (2.5 fold) in 7 D patients than in 157 B patients ($P < 0.01$) (Fig. 1). In total, 3 D and 116 B patients took 5,277 ± 6,461 g and 4,359 ± 5,149 g of Korean red ginseng (KRG) for a prolonged period. For both subtypes, AD was significantly lower in the KRG-treated group than in the KRG-naïve group ($P < 0.05$) (Fig. 2). Excluding patients treated with KRG, AD was significantly more rapid in D patients (2.7 fold) than in B patients ($P < 0.01$), despite similar plasma viral loads. Kaplan–Meier survival analyses of 46 KRG-naïve patients and 164 patients showed a significantly higher mortality rate in D patients than in B patients ($P < 0.001$ and $P < 0.05$, respectively). Based on env gene sequencing for six patients infected with subtype D, all three patients surviving for >10 years were CCR5 virus carriers. In Korea, KRG treatment slowed AD, and progression was more rapid for subtype D infection than for subtype B infection, regardless of KRG treatment.

75. Pemetrexed Inhibits Human Herpesviruses Through Reactivation of P53

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Human herpesviruses have a common virion structure and replication cycle. However, largely due to differences in cell tropisms, the clinical manifestations of infections with different herpesviruses significantly vary. In immunocompromised patients, these viruses are capable of causing severe and sometimes fatal diseases. Current treatments for herpesvirus infections consist mostly of nucleoside analogues, all of which target viral polymerases and are associated with adverse effects and drug resistance. By screening an FDA-approved drug library, we identified pemetrexed as a potent anti-Kaposi's Sarcoma-associated herpesvirus (KSHV) agent with an IC_{50} of 90 nM. Characterization of the antiviral properties of pemetrexed revealed it interferes with replication of viral DNA. The anti-KSHV effect of pemetrexed is dependent on the dTMP synthesis pathway due to its targeting of folate-dependent enzymes, including thymidylate synthase and dihydrofolate reductase. Surprisingly, a new mechanism of action of pemetrexed has been suggested due to the finding that p53 reactivation is strongly related to the anti-KSHV activity of pemetrexed. Further studies found that pemetrexed has broad-spectrum anti-herpesvirus activity against herpes simplex viruses-1 and -2, human cytomegalovirus, and Epstein-Barr virus replication with SIs >1000. In addition, p53 activators also act in an anti-herpesvirus manner by regulating p53 reactivation. Overall, these findings provide strong evidence that pemetrexed inhibits the replication of herpesviruses by regulating p53 reactivation. Thus, our observations support the use of pemetrexed as an anti-herpesvirus agent and p53 reactivation as a novel anti-herpesvirus target.

76. Type I Interferon Induced by a Recombinant Adenovirus Expressing Three Shrnas Enhanced Antiviral Effects against Foot-And-Mouth Disease Virus

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Foot-and-mouth disease virus (FMDV) is the cause of an economically devastating animal disease. The currently available vaccines against foot-and-mouth disease provide no protection until 4-7 days post vaccination. Thus, the only alternative to halt the spread of the FMDV during outbreaks is the use of antiviral agents; combinatorial treatment strategies have been used to enhance the efficacy of antiviral agents, which may be advantageous in overcoming the mechanisms of viral resistance against antiviral treatments. In previous studies, we developed a recombinant adenovirus expressing three short hairpin RNA (shRNA)s that target the non-structural protein-regions, 2B and 3C, of FMDV (Ad-3siRNA). We showed that Ad-3siRNA had a rapid and broad antiviral effect against FMDV *in vitro* and *in vivo*, although shRNA system could have drawbacks such as short duration and reduced effectiveness by viral mutants. In this study, we observed that the antiviral effect of Ad-3siRNA continues up to a minimum of 72 h, and we found that Ad-3siRNA has dual antiviral effects: those of both small interfering RNA and type I interferon. Porcine interferon-ř protein was detected in porcine kidney cells 24h after infection with Ad-3siRNA. In addition, the levels of interferon α/β-stimulated gene (ISG) mRNA in the cells were enhanced 24 h post infection. We believe that this induced type I interferon can be a positive factor for enhancing and increasing the duration of the antiviral effects. We suggest that Ad-3siRNA, which has two different antiviral mechanisms, can be one of the most effective antiviral agents against FMDV.

77. Pomegranate Peel Extracts Possess a Promising Anti-influenza Virus Activity

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Influenza viruses are major causes of acute respiratory infections especially in children and high risk population. They contribute to significant morbidity and mortality worldwide. Limited therapeutic choices are available for treatment and prophylaxis of influenza infections. The emergence of antiviral resistance to currently available influenza antivirals necessitates the search for novel compounds. Polyphenolic compounds present in plants have been reported to possess antiviral activities. Pomegranate (*Punica granatum* L., cultivars) is rich in polyphenolic compounds and previous research have demonstrated the antiviral activity of pomegranate juice. However, the peel which is usually thrown as a waste product was shown to possess the highest concentration of phenolic compounds in pomegranate. In this study, we assessed the anti-influenza virus activity of pomegranate peel extract. The cytotoxicity and antiviral activities of the extracts against influenza were determined on Madin-Darby canine kidney (MDCK) cells. The extracts displayed no cytotoxicity on MDCK cells upon 24 hours of treatment. Treatment of infected cells peel extracts resulted in significant suppression of virus replication. These data demonstrate the anti-influenza virus activity of pomegranate peel extracts and warrants further investigations.

78. Molecular Mechanism of Highly Potent NS5A Inhibitors

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Hepatitis C (HCV) is an enveloped positive-stranded RNA virus that causes chronic in- fections which can develop into liver cirrhosis and hepatocellular carcinoma, locating HCV in a major public health burden. The treatment to combat HCV infection is the use of ribavirin, interferon-alpha and recently, direct acting antivirals (DAAs). DAAs are small molecules targeting non-structural viral proteins which can develop clearing in 98% of in- fection. However, despite advances in recently approved highly potent DAAs the worldwide application of these therapies remains limited due to the expensive cost and potential drug resistance.

NS5A is a multifunctional protein involved in RNA replication, assembly of viral particles and biogenesis of membranous web. It contains an amino-terminal amphipathic alpha-helix (AH) which is responsible for the binding of the protein to the intracellular membranes, a structured domain one (DI) which is involved in the RNA binding and two intrinsically unfolded domains. Here, we show that DAA NS5A inhibitor Daclatasvir (DCV), can block the envelopment of viral particles. Further, we identified Proline residues in NS5A AH-DI structure which are critically involved in RNA replication and might play a role during DCV binding. Additionally, we are running molecular dynamics to investigate what is the role of NS5A inhibitors in a membrane/association context. Taking this together, we aim to develop a structural model that can give an insight of the mode of action of DCV and future NS5A inhibitors in the context of the intracellular membrane. Further results will be presented in poster.

79. Use of Recombinant Herpes Simplex Virus Strains to Characterize Novel UL23 Thymidine Kinase Mutations Toward Resistance to Acyclovir

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Prophylaxis or therapy of herpes simplex virus (HSV) infections with val(acyclovir) ([V]ACV) can lead to the emergence of drug-resistance among 2% to 15% of immunocompromised patients. Alterations in UL23 thymidine kinase (TK) gene of HSV account for 95% of ACV-resistance. However, molecular diagnosis of HSV resistance to antivirals is challenging since the contribution of many mutations remains to be determined. In this study, the role of unknown TK mutations was assessed by the use of a bacterial artificial chromosome (BAC) vector (kind gift from J. Cohen, NIH/NIAID, US): S66P and A72S in HSV-2 TK, and G129R in HSV-1 TK. For HSV-2, both mutations alone or in association were transferred to wild-type UL23 gene BAC vector using directed mutagenesis. For HSV-1, the entire TK gene from the initial clinical isolate harboring the unknown mutation was directly transferred into the BAC vector. Recombinant viruses were generated after BAC transfection into Vero cells. The presence of the desired mutations was validated by UL23 gene sequencing. ACV susceptibility of recombinant viruses was evaluated by plaque reduction assay in comparison to the unmodified BAC-derived HSV strain. Concerning HSV-2 TK, S66P mutation, alone or in association with A72S, conferred ACV-resistance, whereas A72S alone maintained ACV-susceptibility. G129R within HSV-1 TK conferred ACV-resistance. In conclusion, the significance of TK mutations was assessed using recombinant phenotyping: A72S in HSV-2 TK is a natural polymorphism, whereas S66P in HSV-2 TK and G129R in HSV-1 TK have to be considered as ACV-resistance mutations.

80. *In Vitro* Antiviral Activity of Stilbenes Isolated from *Macaranga barteri* against Enteroviruses

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Medicinal plants have always been a credible source of bioactive molecules for the treatment of several human diseases. In recent studies, echovirus 7 and 19 (EV7 & EV19), were identified in Nigeria as disease causing enteric viruses. As there is dearth of antiviral agents against these pathogens, the search for effective antiviral agents is imperative. This study was carried out to search for new antiviral agents from *Macaranga barteri*.

The antiviral activity of three stilbenes; vedelianin, mappain and schweinfurthin G, isolated from *M. barteri,* was investigated in a cell-based assay that estimated the reduction of viral cytopathic effect in human rhabdomyosarcoma (RD) cells. Serial dilutions of the maximum non-toxic dose of each compound was merged with the TCID50 (50% tissue culture infectious doses) of each virus and added to monolayer RD cells in 96-well, flat-bottomed microplates. Treated plates were incubated at 37 °C for 72 h, and the cell viability was evaluated using the MTT (3-[4,5-dimethylthiazol–2-yl]-2,5-diphenyltetrazolium bromide) assay. 50% inhibitory concentration (IC50) of each compound and the selectivity index (SI) was calculated using the GraphPad Prism.

Vedelianin displayed the highest antiviral activity with IC_{50} value of 0.052 and 0.008 nM against EV7 and EV19, respectively. Interestingly, amongst the three stilbenes vedelanin had the highest selectivity for EV19, with a SI value of 31 and 217 against EV7 and EV19, respectively. However, EV7 was resistant to mappain, a compound that displayed a moderate antiviral activity against EV19. These agents may provide templates for the development of new antiviral agents.

81. Antiviral Potential of Fluorinated Analogs of Thymidine

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INTRODUCTION: A considerable increase of the levels of herpetic and adenoviral diseases cause a necessity of the development of effective methods for its prevention and treatment. The fluorinated nucleosides are promising antiviral drugs of our time.

METHODS: The influence of 4-tosyl-5-polyfluoroalkyl-1,2,3-triazoles (G16, G18, G19, and G29) on the infectivity of herpes simplex virus type 1 (HSV-1) and human adenovirus type 5 (HAdV-5) was carried out with MTT assay and cytomorphology method. Influent of compounds on the cell cycle under a condition of adeno- and herpesvirus infections was studied using flow cytometric analysis of propidium iodide-stained cells.

RESULTS: From four analogs of thymidine, only 2-(3-clorotetrahydrofuran-2-yl)-4-tosyl-5-heptafluoropropil-1,2,3-triazole (G29) suppressed HAdV-5 and HSV-1 reproduction by 50% in concentrations of 37μg/ml and 10 μg/ml, respectively. The G29 reduces the titer of viruses obtained *de novo* and inhibited HSV-1 and HAdV-5 inclusion-bodies formation by 84 – 90% and 71 – 83%, respectively. It is known that the transition of the cells from G1 to S phase and arrest of G2 phase cell cycle is mandatory for lytic replication of a number of viruses. The normalization of the number of cells in all phases of the cell cycle compared with the profile of infected cells and the decreasing number of the apoptotic cells by 27 – 73% compared with the control values of viral infections were determined after using of G29.

CONCLUSION: The fluorinated G29 showed potent activity against HAdV-5 and HSV-1; it is needed further investigation to the detailed mechanism of antiviral action of the compound.

82. Induction of Apoptosis in the EBV-Associated Cell by Fluorinated Derivatives

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Apoptosis plays an essential role in the development and maintenance of all mammalian tissues. Defects in apoptosis mechanisms play important roles in tumor pathogenesis, such as EBV-associated limphoproliferative disorders. The past decade, the fluoride analogs are of a special interest.

The aim of the study was to evaluate the ability to induce apoptosis of fluorinated analogs using *in vitro* and *in silico* approaches. Fluorinated derivatives of uracil (G27), tiosugars (SBIO6), amino acids (10S21) were synthesized in Institute of Organic Chemistry NAS of Ukraine. The research was carried out by using flow cytometry, fluorescent microscopy, PCR, and PharmMapper software.

Possible targets for all compounds were identified. It was established, that majority of targets are enzymes that participate in the apoptotic cascades, such as MAPK14, Apaf1, cell division protein kinase and other. Anti-EBV activity was observed for G27, SBIO6 and 10S21 and EC_{50} values were 100, 67 and 1μg/ml on Raji cell. The ř95-8 and Raji cells treated with all compounds were analyzed. It was marked an induction of apoptosis in the presence of SBIO6, at 250μg/ml of which, the portion of apoptotic cells reřched almost 90% on B95-8 and nearly 60% on Raji cells. Compound G27 was less effective, the percentage of apoptotic cell reached 50% on both cell lines. Compound 10S21 induced apoptosis more effective on Raji cell line (52%) than on B95-8 cells (42%).

This study of fluorinated derivatives had shown the ability to induce apoptosis at B-lymphomas that open new directions in the study of this class compounds.

83. Initial Studies on T-1105's Redox Properties and Its Structural Similarity to Nicotinamide

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T-1105, the non-fluorinated analogue of the antiviral T-705, exhibited favorable antiviral properties in cell-based studies. Moreover, chemical analyses revealed T-705's profound lability when linked to ribose; in contrast, T-1105-ribonucleoside proved much more stable in aqueous, buffered solution. The activating metabolism of the pyrazinecarboxamide parents starts by HGPRT-catalyzed phosphoribosylation. From numerous experiments, it was concluded that, owing to their rotatable carboxamide moiety, both pseudobases mimic the natural purine bases.

Based on their chemical structure, an analogy of the pyrazinecarboxamides with nicotinamide is proposed. Known examples underlining this hypothesis are Tiazofurin and benzamide riboside; both are converted to the NAD analogues TAD and BAD intracellularly. To study this analogy, e.g. challenge enzymes of the nicotinamide metabolism with potential T-1105-modified substrate analogues, we synthesized T-1105-Adenine-Dinucleotide via the cycloSal-technique. Further, we analyzed the redox properties of T-1105, T-1105-ribonucleoside and its 5'-monophosphate. Methods used included UV/Vis- and NMR-spectroscopy and the results demonstrated that the pyrazinecarboxamide's reduction was reversible. Reduction involved the loss of aromaticity, and consequently, the overall absorption intensity decreased upon addition of a reducing agent. The kinetics of this reaction proved fastest for the unmodified pseudobase. Upon addition of an oxidizing agent to the reaction mixture, the absorption at $lambda_{max}$=345 nm (base), or 350 nm (riboside) was restored. Further chemical and enzymatic studies are ongoing.

84. Acyclovir-Resistant Herpetic Keratitis (HK) in an Immunocompetent Patient

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HK caused by herpes simplex virus 1 (HSV-1) remains one of the leading causes of infectious corneal blindness worldwide. Valacyclovir (VACV), the oral prodrug of acyclovir (ACV) constitutes the first-line drug with high antiviral efficacy. However, ACV-resistance may impair drug efficacy.

We report here a case of a 43-year-old man with a long history of recurrent epithelial HK successfully cured over past decades. On October, 2017, he presented with ocular redness and decreased vision. Dendritic HK was diagnosed and samples collected for virological investigation were positive for HSV-1. After corneal epithelial debridement, he was treated with oral VACV and topical corticosteroid. Since clinical symptoms remained unchanged, antiviral therapy was modified with successive topical adjunction of ganciclovir and trifluorothymidine. Despite well-conducted treatments, new dendritic lesions appeared raising the suspicion of ACV-resistance. As corneal scrapping was still positive for HSV-1, full-length thymidine kinase (TK) and DNA polymerase viral genes were amplified and sequenced for genotypic antiviral resistance testing. Apart from natural polymorphisms, the isolate harbored an amino acid change unpreviously described within TK and potentially conferring ACV-resistance. Indeed, this novel change L340R was associated with ACV-resistance phenotype using plaque reduction assay in cell culture. Retrospective study showed that L340R change appeared under antiviral selection pressure. Intravenous foscarnet treatment was started and healing occurred after 4 days. This case highlights the possible emergence of ACV-resistance among immunocompetent patients with recurrent HK. Antiviral resistance has to be promptly detected in order to switch treatment to avoid corneal morbidity associated with recurrent HK.

85. Human SAMHD1 Restricts the Xenotransplantation Relevant Porcine Endogenous Retrovirus (PERV)

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INTRODUCTION: The Transplantation of porcine cells, tissues, or organs into human recipients may provide a means to alleviate the shortage of human transplants. Porcine endogenous retroviruses (PERVs) are a potential risk factor in xenotransplantation because PERV particles are released from pig cells and infect human cells.

Human SAMHD1 starves reverse transcription of retroviruses by depleting the cellular dNTP pool in monocytes, macrophages, dendritic cells and resting $CD4^+$ T cells. The VPX accessory protein from HIV-2 and some SIVs degrades SAMHD1 allowing for the dNTP pool to rise and reverse transcription to occur.

OBJECTIVE: This study aims to understand the role of the restriction factor SAMHD1 as a transmission barrier for PERV-containing pig donor organs to the human recipient.

RESULTS: The qPCR results showed that human MDDC, MDM and monocytes restrict PERV reverse transcription similar to that of HIV-1. Addition of VPX-VLP increases the amount of PERV reverse transcripts in the target cells by at least 4-fold in all donors MDDC tested and at least 10-fold in MDM and monocytes, suggesting a SAMHD1-mediated restriction. Addition of deoxy nucleoside (DN) to the culture medium of dendritic cells increased PERV cDNA in the absence of VPX. In addition, THP1 cells with a stable CRISPR/Cas9 mediated SAMHD1 KO supported PERV reverse transcription in contrast SAMHD1-expressing THP1 cells.

CONCLUSION: These results suggest that human SAMHD1 is a restriction factor to PERV. Thus, in addition to APOBEC3s and tetherin, human SAMHD1 provides a barrier for PERV transmission during xenotransplantation from pig organs to human recipients.

86. Biological Evaluation of Novel Small-Molecule Antiviral Agents Versus Chikungunya Virus

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus causing flu-like illness in the human host, with severe long-lasting arthralgia and therapy-resistant headaches, but only rarely meningoencephalitis. There have been several significant outbreaks in recent years. So far, there are no CHIKV specific vaccines or therapeutics.

Starting from the structures of antiviral hits that have previously been identified with *in silico* techniques (Basetto et al., 2013, Tardugno et al., 2018), 19 novel structural analogues were designed and prepared. Activity versus CHIKV was tested in simian VeroB4 cells, where CHIKV causes cytopathogenic effects (CPE), but only limited cell death, presumably due to viral gene products inhibiting apoptosis (Joubert et al., 2012). This phenomenon was even more pronounced in HUH7 human hepatoma and DBTRG human brain glia cells.

An initial ranging assay at 10uM revealed several compounds with IC50 <= 10uM and minimal toxicity in VeroB4 cells. Further biological evaluation of these compounds in different cell lines, including more sensitive HUH7 cells (high selective index in HUH7 cells > lead), as well as characterization of lead compounds in brain cells and different assay methods is ongoing and will be reported.

This work provides the foundation for further investigation of promising novel structures as antiviral agents against Chikungunya virus.

87. Design of Effective Multivalent Influenza Virus Inhibitors Preventing Binding to Host Cells

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The past influenza season reveals how vulnerable human mankind still is. Vaccination is one possibility to get protection against the high mutable virus. Using the principle of multivalency, the mechanism influenza binds to its host cell, is another strategy we are following. Binding is mediated by hemagglutinin (HA) that forms a densely packed layer of homotrimeric organized spikes on the virus envelope. Virus binds via HA with high affinity to sialic acid (SA) moieties of the host cells glycocalyx by multivalent interactions. Displaying multiple HA receptors on an appropriate scaffold to mimic host cell membrane is one promising strategy to prevent influenza binding to host cells and, thus, infection.

Using constructs of either a DNA-PNA complex coupled to a 6'-sialyl-LacNac displaying a defined spatial ligand arrangement or polyglycerol based Nanogels with more randomly placed SA ligands we surmise that not only the spacing between two ligands is important for binding even the arrangement of a sum of ligands, leading to the conclusion that inhibition efficiency is likely depending on whether the virus binds HA inter- (between two HA trimers) or intratrimeric (within one HA trimer).

Here we presenting different multivalent inhibitor architectures tending either to inter- or intratrimeric binding. Inhibition potential was assessed using hemagglutination inhibition assay. A549 cell line based viability studies demonstrate efficient inhibition of influenza virus X31 infection. Furthermore, constructs and virions were imaged using Cryo-EM. Comparison of the results of the different constructs provide new insights on the mechanism of HA binding to effective multivalent inhibitors.

88. Identification of Novel Norovirus Antiviral CMX521 Using High-Throughput Screening

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There are currently no FDA-approved vaccines or antivirals available for treatment or prophylaxis of human norovirus. The discovery of broad-spectrum norovirus antivirals has been historically hindered by the lack of good *in vitro* culture systems for human norovirus (HuNoV) and the genetic diversity of Norovirus. Since the active site of the norovirus RNA polymerase shows less diversity than other regions (93% amino acid homology), even across species, we reasoned that mouse norovirus (MNV) could be used to identify nucleoside antivirals which should possess pan-norovirus activity.

A novel MNV was isolated from mouse feces, characterized, and used to screen a library of ~3500 nucleoside analogs. This cell culture-adapted MNV isolate was highly cytolytic; lysis was observed in mouse macrophage-derived cells (RAW 264.7) within two days after infection making it ideal for a rapid high-throughput screen. Compounds showing activity in 3-shot screens were confirmed in a full dose-response MNV assay. Cytotoxicity was measured using MTS- or Cell Titer-Glo-based endpoints in human T-cell derived cells (MT4) and RAW cells. Anti-HuNoV activity was confirmed in a HuNoV replicon assay that utilized an RT-qPCR endpoint to quantitate RNA levels. Of the 89 hits identified in the screen, CMX521 had the best activity/toxicity profile, producing a mean +/-SD EC50 of 1.9 +/- 0.8 μM (n=69) in the MNV assay and a mean +/-SD EC50 of 1.61 +/- 0.66 μM (n=27) in the HuNoV replicon assay. Additional assays have revealed broad activity across all MNV and HuNoV strains tested. CMX521 has progressed to Phase 1 clinical development.

89. Synthesis and Evaluation of Tenofovir Diester Prodrugs as Potential Long-acting Agents for Treatment and Prevention of HIV-1 Infection

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Longer-acting antiretroviral drugs for treatment and prevention of HIV-1 infection are of interest for their potential to improve patient adherence and virologic control. To improve the drug-delivery profile of tenofovir (TFV), we designed the prodrug approach illustrated in Figure 1, incorporating a long-chain alkoxyalkyl group (**R_1**) to promote rapid intracellular uptake, and a more stable promoiety (**R_2**) that slows the activation rate to maintain effective levels of the active metabolite, tenofovir diphosphate (TFVpp). We reported previously that octadecyloxyethyl-benzyl-tenofovir (ODE-Bn-TFV) exhibited potent inhibition of HIV-1 replication (EC_{50} = 4 nM) and showed evidence of prolonged cell retention.

To evaluate other **R_2** promoieties for improved sustained release, we synthesized additional TFV diesters, including various alkyl, cycloalkyl, heteroalkyl, substituted benzyl and aryl esters and assessed anti-HIV activity in PBMCs. Among the diesters, octadecyloxyethyl-ethyl-TFV (ODE-Et-TFV) exhibited less potent, but still significant, inhibition of HIV-1 replication (EC_{50} = 31 nM) which may indicate slowed activation and a potentially longer-acting pharmacokinetic profile. Substituted benzyl and aryl **R_2** promoieties showed activity comparable to the lead compound, ODE-Bn-TFV.

Hoping to demonstrate intracellular prodrug accumulation and prolonged maintenance of TFVpp levels, we exposed human foreskin fibroblast (HFF) cells to several new analogs, washed, and then cultured them for varying times prior to LC/MS analysis. ODE-Bn-TFV treatment resulted in measurable TFVpp for 28 days, whereas ODE-phenyl-TFV TFVpp levels were slightly lower, but also long-lasting.

Enhanced cellular uptake and retention of lipophilic TFV diesters such as ODE-Bn-TFV may provide long-lasting suppression of HIV-1 replication and could improve HIV-1 treatment and prevention.

90. First Steps Towards Developing Novel Inhibitors of Enterovirus Polymerases

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Enteroviruses are responsible for several diseases like poliomyelitis, hand-foot-and-mouth disease, aseptic meningitis and myocarditis. Currently, no antiviral drugs targeting enteroviruses are available on the market.

A novel broad-spectrum non-nucleoside inhibitor targeting enterovirus polymerases was described while requiring improvements for further development. This study aimed to identify new chemical scaffolds retrieving the interaction with the biological target, enterovirus polymerases, with improved drug-like properties.

To this end, 2000 drug fragments from the Prestwick Drug-Fragment Library have been virtually combined to create numerous chemical structures, mainly compliant with established medicinal chemistry rules.

A structure-based drug design approach was initiated with the crystal structure of the polymerase from Coxsackievirus B3: A pharmacophore model was built to contain the relevant features responsible for the biological activity, using the LigandScout software.

This model was then used for virtual High Throughput Screening (vHTS): Potential bio-active molecules were retrieved from the database of combined drugs fragments. Relevant structures were selected from this hit list. The use of related and shared starting materials enabled to obtain various scaffolds with a limited number of reactions.

Synthesized compounds were evaluated by *in vitro* experiments against two strains of picornavirus. Besides investigation of new scaffolds, hit validation and derivatisation of most promising hits can afford new potential anti-enterovirus leads.

This project applies computational chemistry techniques, associated with an innovative use of fragments to optimize the hit discovery process.

91. Identification of Natural Product-Based Inhibitors Targeting Enterovirus 71 VP1-Receptor Interaction

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Our laboratory focuses on identifying potent anti-enterovirus A71 (EV-A71) compounds from synthetic chemical libraries or natural products and studying their underlying antiviral mechanisms. CGU-V007 is a natural product purified from a herbal medicine. Results of the time-of-addition assay suggest that CGU-V007 affects an early stage of virus infection. Further experiments demonstrated that CGU-V007 might target viral particles directly, thereby interfering with virus-receptor interactions. Consistently, sequencing of the plaque-purified CGU-V007-resistant viruses showed two mutations in the nonstructural protein VP1, P246A and E98G located near the five-fold axis of EV-A71, which is associated with virus-PSGL1 and virus-heparan sulfate interactions. Since the VP1 E98G and P246A residues are conserved in EV-A71 strains, we proposed that CGU-V007 could potentially inhibit EV-A71 infections of emergent variants.

92. Evaluation of Severe Disease Mouse Model Caused by Highly Pathogenic Avian Influenza Infection

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Highly pathogenic avian influenza (HPAI) infection known to be responsible for severe clinical complications such as hyper-cytokinemia (HC), hypertriglyceridemia (HGA) and splenomegaly in human. However, the complications have not been well defined in animal model. In this study, we observed the complication caused by HPAI in mice. DBA/2J mice were infected with HPAIs [A/VietNam/1194/2004 (VN) or A/Broiler duck/Buan2/2014 (BA)] and the complications and mortality were investigated. Mice mortality was increased dose dependent manner after both viruses infection. Pro-inflammatory cytokines/chemokines (IFN-ř, IL-6, TNF-ř, RANTES and MIP-1ř) increased up to the 90-fold. The splenomegaly was observed in 1 and 100 mLD50 of VN and 1mLD50 of BA, showing 10% increased spleen length, compared with the other mice. The HGA was also induced by 1 and 10 mLD50 of VN. These suggested that HPAI infections cause the severe complication in the mice. This results would provide useful mouse model information for us to evaluate antiviral reagent. This research was supported by a fund by Research of Korea Centers for Disease Control and Prevention (Grant number: 2016-NI43001-00).

KEY WORD: Highly pathogenic avian influenza, hypercytokinemia, hypertriglyceridemia, hemophagocyticlymphohistocytosis

93. Characterization of Murine Gammaherpesvirus-68 (MHV-68) Resistance and Fitness

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Previous studies concerning murine gammaherpesvirus-68 (MHV-68) antiviral drug-resistance reported resistance to nucleoside analogs, including licensed antivirals as well as novel inhibitors of herpesvirus replication. Despite progress in understanding the mechanisms of MHV-68 resistance to antiviral agents, no information is currently available regarding resistance to nucleotide or pyrophosphate analogs. Besides this, the emergence of drug-resistant viruses raises the question of the impact of mutations on the viral replication capacity.

In this study, we elucidate the mechanism of antiviral drug-resistance to various nucleoside, nucleotide and pyrophosphate analogs. After selection, we genotype the drug-resistant viruses and determine the drug-susceptibility profile. Subsequently, we evaluate the replication capacity of drug-resistant viral strains in competition with wild-type virus, both with and without antiviral pressure. The study includes newly and previously obtained MHV-68 resistant viruses carrying mutations in the viral thymidine kinase, protein kinase and DNA polymerase.

Under pressure of ganciclovir (GCV), cidofovir (CDV), HPMP-5azaC or foscarnet (PFA), we characterize 6 mutations associated with drug-resistance. All mutations are located in the viral DNA polymerase [GCV^R-MHV-68 (Y383S), CDV^R-MHV-68 (C981Y), HPMP-5azaC^R-MHV-68 (Q827R), PFA^R-MHV-68 clone 1 (G302W+K442T), PFA^R-MHV-68 clone 2 (G302W) PFA^R-MHV-68 clone 3 (K442T)]. We show that the acquired viruses are resistant to the selecting antiviral and that cross-resistance occurs. We demonstrate that in competitive fitness studies without drug-pressure, MHV-68 resistant viruses are equally or less fit compared to the wild-type virus and generally, under drug-pressure there is a shift in the composition of the viral population to a 3- to 4-fold increase in the proportion of drug-resistant virus.

94. Targeting Cyclophilin A to Block Viral Innate Immune Evasion and Engage Antiviral Immune Responses

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Cyclophilin A (CypA) is required for many viral infections. We previously showed that CypA stabilises the HIV-1 capsid, preventing premature uncoating to help HIV-1 evade cytosolic innate immune sensors. Treatment of infected macrophages with cyclophilin inhibitors (CypI) elicited interferon (IFN)-ř production and suppressed HIV-1 replication. For HCV, CypI were similarly shown to suppress viral replication and increase IFN expression in chronically infected patients, although the mechanisms are unclear. CypI disrupt formation of the membranous HCV replication complex (RC), which is thought to cloak viral replication intermediates from innate immune sensors. We hypothesised that CypA contributes to HCV innate immune evasion by modulating RC formation. We employed chemical biology to dissect the role of CypA in HCV innate immunity. Cells were infected with HCVcc (Luc-J6/JFH1) or electroporated with HCV subgenomic replicon (Luc-JFH1), which forms the RC and replicates viral RNA. After 4h, a panel of novel CypI were added and HCV luciferase reporter activity was measured 48h-72h later. Active CypI were ~10-fold more potent in Huh7 cells (with intact RNA sensing) than in Huh7.5 cells (with defective RNA sensing due to a RIG-I mutation). The potent anti-HCV activity (EC_{50}, ~100 nM) in Huh7 cells corresponded to an induction in IFN-ř expression, which was lacking in Huh7.5 cells. We are currently characterising the specific mechanisms, as well as extending our findings to other CypA-dependent flaviviruses (e.g., Dengue). Overall, this work suggests a novel antiviral paradigm, whereby inhibiting viral evasion strategies engages innate immune sensing and enrolls host immune responses to clear infection.

95. High-Throughput Screening and Development of Antiviral Compound against Middle East Respiratory Syndrome–Coronavirus (MERS-Cov)

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Middle East respiratory syndrome-coronavirus (MERS-CoV) causes severe lower respiratory tract infection in humans. Since the first identification at Saudi Arabia in 2012, the virus has led to 2,143 patients and 750 deaths worldwide until February 2018 (mortality rate, 35%). In particular, a large outbreak in Korea resulted in 186 patients and 38 deaths in 2015. Shortly afterwards Gyeonggi province where the epidemic had been initiated, launched a project to develop antiviral drugs to prevent the recurrence of MERS outbreak.

In this study, we set up several screening methods i) Pseudovirus infection assay, ii) AlphaScreen™ iii) Papain-like protease (PLpro) inhibition assay and iv) MERS-CoV infection assay. We carried out high-throughput screening (HTS) using 30,000 in-house chemical library. Compounds inhibiting earlier steps of MERS-CoV infection *i.e.* receptor binding, internalization or endosomal trafficking, could be screened by the pseudovirus infection assay. Direct inhibitors of the interaction between spike and Dipeptidyl peptidase 4 (DPP4) were selected by the AlphaScreen™. We verified 6 positive scaffolds by cell-based MERS-CoV infection assay. PLpro inhibitors were also screened and 9 different scaffolds were proved to reduce MERS-CoV propagation. Among the hits, we picked up prominent ones and are in the middle of optimizing lead compounds awaiting animal studies with hDPP4 transgenic mice.

96. Preliminary *In Vitro* Antiretroviral Activity of a *Nigella Sativa* Seed Formulation (A-Zam) against HIV-1

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Unavailability of the highly active antiretroviral therapy (HAART) to the teeming population living with human immunodeficiency virus type 1 (HIV-1) infection in the Western and Central Africa means that they have to seek an alternative treatment option. Therefore, traditional herbal medicine seems to be a common option. This study was aimed at validating the efficacy of such a remedy, A-zam, against HIV-1 *in vitro*. A-zam was examined for its anti-HIV-1 activity and cytotoxicity in acutely and chronically infected cells. The anti-HIV-1 activity was determined by the inhibition of cytopathic effect in acutely infected MT-4 cells using the MTT method, while it was determined by the inhibition of p24 antigen production in chronically infected OM-10.1 cells using ELISA. The cytotoxicity of A-zam was also determined by the MTT method in the chronically infected cells. A-zam did not show anti-HIV-1 activity in acutely infected cells. The cells displayed definitive cytopathic effect and only 23.9% - 24.9% survived at 250-6250 fold dilutions of the drug. Interestingly, Alpha-zam selectively inhibited the p24 antigen production in OM-10.1 cell after

stimulation with TNF-ř. The highest inhibition (84.6%) was achieved at the 100-fold dilution, suggesting that A-zam may have a potential anti-HIV-1 activity in chronically infected cells. The results of the present study appear connected with results in previous human study, where decrease of plasma viral load, increase of $CD4^+$ T-cell count and improved quality of life were observed in patients. These results suggest that A-zam may be a good candidate for alleviating immunosuppressive conditions like HIV-1/AIDS.

97. A High Content Imaging-Based Neutralization (HINT) Assay to Assess Susceptibility of Influenza Viruses to Antivirals Targeting Virus Entry

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In recent years, substantial efforts were made to improve therapeutics options by developing new antiviral agents. Lectins, carbohydrate-binding proteins, are capable of preventing influenza infection by binding to HA and interfering with its receptor-binding and membrane-fusion function. Antiviral lectins predominantly recognize high-mannose glycans. The complexity of HA glycosylation can differ when virus replication takes place in human respiratory tract epithelial cells or laboratory cell lines Therefore, in the present study we sought to investigate the susceptibility of influenza viruses to antiviral lectins in human respiratory specimens and influenza viruses isolated in laboratory cell culture (i.e. MDCK-SIAT1). Isolates or clinical specimens were pre-incubated with serially diluted lectins and then added to cells to allow infection. in absence of trypsin. To determine IC50, individual virus-infected cells were immunostained using a combination of fluorescent dyes and counted using a high-content imaging platform (CellInsight). Clinical specimens containing influenza A(H3N2) viruses (with Ct values <25), submitted for virological surveillance during the 2017-2018 season were used to assess susceptibility to cyanovirin-N (CV-N) and banana lectin (BanLec, *Musa paradisiaca*). The IC50 values for CV-N and BanLec were 1.1 nM and 3.2 nM, respectively. The values were similar to those determined for their matching MDCK-SIAT1-grown isolates. Notably, 2 of 20 tested A(H3N2) viruses showed a 3-fold reduced susceptibility to BanLec and contained the amino acid substitution T135K, which resulted in the loss of a glycosylation sequon at Asn 133. Testing of seasonal and emerging zoonotic influenza viruses with the engineered lectin BanLec H87T is underway.

98. Laboratory and Clinical Strains of Dengue Virus Differentially Affect Endothelial Cells

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Dengue, caused by four dengue virus serotypes (DENV 1-4), is the most prevalent arthropod-borne viral disease in humans. Increased vascular permeability and plasma leakage are the hallmarks of severe dengue, but the extent to which endothelial cells (ECs) actively contribute to DENV pathogenesis remains debatable. Here, we characterized the responses of different EC types to infection with laboratory strains ($DENV_{lab}$) and clinical isolates ($DENV_{clin}$). The microvascular EC line HMEC-1 showed the highest susceptibility to $DENV_{lab}$ 1-4, whereas infection of primary ECs resulted in a lower infection rate. The infection rates correlated with cell surface expression levels of heparan sulfate (HS) and were also associated with increased apoptosis. Using the Bio-Plex 200 system, we detected a strong upregulation of cytokines involved in the regulation of endothelial permeability and immune cell activation/recruitment. The secretion of cytokines did not strictly relate to the degree of infection, but showed to be strain-specific. $DENV_{clin}$ isolates showed similar infection rates in different EC types, indicating that they are less dependent on HS expression. Moreover, while DENV-3 was the least infectious laboratory strain, it was by far the most infectious clinical strain in all ECs tested. In contrast to the other clinical strains, the $DENV_{clin}$-3 did not induce caspase-3 activation nor apoptosis in infected ECs. Our data indicate that EC responses are mainly determined by the infecting viral type, and by the degree of adaptation of the virus to cell culture, suggesting that low passage, clinical isolates of DENV should preferentially be used to study DENV pathogenesis.

99. Divide Et Impera, Small Molecule Disruptors of Ul44 Dna Polymerase Processivity Factor: A New Class of Cytomegalovirus Inhibitors

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Human cytomegalovirus (HCMV) is a leading cause of severe disease in immunocompromised individuals, including AIDS and transplanted patients, and in congenitally infected newborns. Despite the availability of several drugs, pharmacological treatment of HCMV infections is associated with poor bioavailability, toxicity and the emergence of resistant strains. Therefore, it is essential to identify new potential targets of therapeutic intervention. Dimerization of HCMV DNA polymerase processivity factor UL44 plays an essential role in the viral life cycle being absolutely required for DNA binding and Ori*Lyt*-dependent DNA replication.

We therefore validated the recently published crystal structure of UL44 homodimers both *in vitro* and in a cellular context by a variety of assays, our results showing that single amino acid substitutions at the dimerization interface strongly affect UL44 dimerization. Subsequently, we used such structure to identify 140 small molecules (SMs) potentially interfering with UL44 homodimerization, by means of an *in silico* screening. In depth characterization of 18 selected SMs candidates led to the identification of four compounds capable of inhibiting the replication of different HCMV strains with ED50s in the low micromolar range. Clearly, our data demonstrate that SM disruptors of UL44 dimerization represent a new class of highly needed HCMV inhibitors, alternative to those targeting the DNA polymerase catalytic subunit or the viral terminase complex.

100. Development and Characterization of a Recombinant Lassa Virus Expressing Enhanced Green Fluorescent Protein as a Tool for High-throughput Drug Screen and Neutralizing Antibody Assays

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Lassa virus (LASV), a member of the family *Arenaviridae*, is a highly virulent National Institute of Allergy and Infectious Diseases Priority, US Select Agent, and Risk Group 4 Pathogen. LASV infects an estimated 300,000–500,000 individuals yearly in Western Africa and is associated with high morbidity and lethality. Currently, no FDA-approved antiviral drugs or vaccines are available for prevention or treatment of LASV infection. Developing screening platforms for antiviral compound discovery is crucial to generate medical countermeasures (MCMs) to counter viral infection. Using reverse genetics, we generated a wild-type recombinant LASV isolate Josiah (rLASV-wt) and modified version thereof encoding a cleavable enhanced green fluorescent protein (eGFP) as a reporter (rLASV-eGFP). Using two MOIs and two cell lines, we found the viral growth kinetics of rLASV-wt to be similar to wild-type LASV, whereas rLASV-eGFP was slightly attenuated. eGFP expression of rLASV-eGFP reporter expression is stable for at least six passages. Using the two well-characterized LASV inhibitors favipiravir and ribavirin, we demonstrate that rLASV-eGFP is suitable as a tool for the identification of inhibitors against wild-type LASV. Therefore, we established a rLASV-eGFP-based high-throughput drug discovery screening platform and a rLASV-eGFP virus-based neutralization assay for LASV-neutralizing antibody screening. These platforms will be used to accelerate MCM discover and reduce costs of antiviral screens in biosafety level 4 (BSL-4) containment.

101. Kinetic, Thermodynamic and Structural Analysis of the H275Y, I223V, and S247N Neuraminidase Resistant Mutants of the 2009 Pandemic Influenza Virus

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Influenza is a major human pathogen. Neuraminidase (NA), which plays an indispensable role in the last step of viral cycle, is the main influenza drug target. To date, this class of antivirals encompasses four neuraminidase inhibitors with oseltamivir being the most widely prescribed. However, drug resistant viruses emerge readily. Neuraminidase major oseltamivir resistance mutation H275Y alone or in combination with a substitution I223V and S247N have been observed in the 2009 pandemic H1N1 viruses („swine flu").

To evaluate the influence of these mutations on pandemic NA2009 sensitivity to oseltamivir, we overexpressed and purified the ectodomain of the wt neuraminidase from the influenza virus A/California/07/2009 (H1N1) as well as recombinants containing H275Y, I223V, and S247N single mutations and the H275Y, I223V and H275Y, S247N double mutants. We enzymologically characterized these enzymes with oseltamivir and performed thermodynamic analyses of oseltamivir binding to neuraminidase using isothermal titration calorimetry. Finally, to decipher structural basis mechanism of resistance we determined crystal structures of mutated NA2009 enzymes in complex with oseltamivir carboxylate. We found that the I223V or S247N single amino acid substitution confers only moderate reduction in oseltamivir affinity to the NA2009. On the other hand, the major mutation H275Y causes significant reduction in the ability to bind this drug and its combination with I223V or S247N mutation leads to the substantial impairment of oseltamivir inhibition potency. Our structural analyses uncovered major structural effect of H275Y substitution on oseltamivir pose and synergic effect on resistance caused by combination of H275Y with S247N and I223V substitution.

102. Combination of Enterovirus Inhibitors Delay or Prevent the Development of Enterovirus-A71 Resistant Variants

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There are no antivirals available to treat infection with enterovirus A71 (EV-A71) or any other enterovirus. The extensively studied capsid-binders select rapidly for drug-resistant variants. We here explore whether the combination of two direct-acting anti-enterovirus molecules with a different mechanism of action may delay or prevent resistance development. To that end the *in vitro* dynamics of resistance development to the capsid-binder pirodavir was studied either alone or in combination with (i) the viral 2C-targeting compound SMK_0213, (ii) the viral 3C-protease inhibitor rupintrivir or (iii) the viral polymerase inhibitor 7-deaza-2'*C*-methyladenosine (7DMA). RD cells infected with EV-A71 were treated with sub-optimal concentrations [1xEC_{50}] of either pirodavir alone or in combination with one of the other compounds. Culturing the virus at the 1xEC_{50} pirodavir resulted already after 2 passages in a shift in sensitivity to the compound. When 1xEC_{50} pirodavir was combined with 1xEC_{50} rupintrivir, resistance to pirodavir developed later (passage 4) and no resistance to rupintrvir, was recorded at the end of the experiment (passage 8) either in the combination or rupintrivir alone. When 1xEC_{50} pirodavir was combined with 2xEC_{50} (but not 1xEC_{50}) of SMK_0213 delayed resistance development to pirodavir was noted. When 1xEC_{50} pirodavir was combined with 1xEC_{50} 7DMA resistance did not develop to either compound up to passage 4.

Thus, combinations of enterovirus inhibitors with different mechanism of actions (in this study entry and non-entry inhibitors) delays the development of resistance *in vitro* and may circumvent the problem of rapid emergence of resistance to capsid-binders *in vivo*.

103. A New System for a Silent Virus: Developing a Skin Tissue Model for Human Cytomegalovirus

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Human cytomegalovirus (HCMV) infects >50% of individuals. HCMV is usually asymptomatic, but the virus remains in the body and can reactivate. If a pregnant woman reactivates or is re-infected, HCMV can infect the fetus. Unfortunately, HCMV is the leading cause of congenital birth defects, and is a serious problem in transplant patients. Treating HCMV is complicated by the few drugs available and their toxicity. Using an HCMV strain expressing luciferase from a late viral promoter and eGFP, we evaluated growth *in vitro* and in skin-organ culture (SOC). We expected that bioluminescence would indicate HCMV growth kinetics and fluorescence would provide a visual marker of infection. Bioluminescence from HCMV-infected cells was measured by IVIS (*In Vivo* Imaging System), and eGFP fluorescence was measured by microscopy and flow cytometry. In cultured cells, the amount of virus inoculum was proportional to bioluminescent and fluorescent signals, as well as cytopathic effects. Bioluminescence was also significantly correlated with GFP+ cells. Notably, production of infectious HCMV occurred several days after bioluminescence and fluorescent signals were detected. In SOC, HCMV-infected cells were detected over 14 days using IVIS, and HCMV foci were observed by fluorescent microscopy. Additionally, effects of antiviral drugs were tracked using IVIS. Particularly, cidofovir and foscarnet prevented HCMV infection. Letermovir and ganciclovir testing in SOC is ongoing. Establishing an efficient model for HCMV replication in human skin tissue will be clinically relevant to further understand viral growth kinetics and study antiviral effects.

104. Inhibition of Cytosolic Phospholipase A2alpha Impairs Coronavirus Replication by Interfering with Virus-Induced Replicative Organelle Formation

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Similar to other +RNA viruses, coronaviruses induce membrane rearrangements in infected cells, resulting in organelle-like 'virus factories' that carry multi-subunit complexes that drive viral RNA synthesis. There is increasing evidence that enzymes involved in cellular lipid metabolism have important roles in RO formation and other steps of the coronavirus replication cycle. In this study, we assessed the role of cellular cytosolic phospholipase A2alpha (cPLA2a) activity in the replication of corona- and other RNA viruses. cPLA2a catalyzes the hydrolysis of membrane-associated glycerophospholipids at the sn-2 position, releasing a fatty acid and generating a lysophospholipid (LPL). In human coronavirus 229E (HCoV-229E)-infected Huh-7 cells, inhibition of cPLA2a activity was found to impair viral protein and RNA accumulation and resulted in reduced numbers of ROs. Furthermore, inhibition of cPLA2a activity resulted in reduced LPL levels, suggesting an involvement of LPLs in producing the membranous structures required for coronavirus replication. This is further supported by lipidome analyses of HCoV-229E-infected (compared to mock-infected) cells, which revealed an increase of LPLs in virus-infected cells. This increase is suppressed in the presence of cPLA2a inhibitor. Finally, we were able to confirm that inhibition of cPLA2a activity affects the replication of several other +RNA viruses known to induce intracellular membrane rearrangements, such as MERS-CoV and Semliki forest virus, whereas poliovirus, human rhinovirus 1A, and vaccinia virus replication was not affected. Taken together, the data provide strong evidence to suggest that cellular cPLA2a activity has important roles at different steps of the replication cycle of +RNA viruses from different families.

105. The Search for Novel Zika Virus Inhibitors and the Establishment of Relevant *In Vivo* Mouse Models to Evaluate their Efficacy

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The rapid geographical spread of Zika virus (ZIKV) poses a serious public health concern given the dramatic upsurge in the number of microcephaly cases that was noted in newborns born to mothers infected with ZIKV. Although ZIKV is an arthropod-borne virus, sexual transmission of ZIKV has gained more attention during the latest outbreak in the Americas, with several reports on sexual transmission[1]. Currently, a vaccine or antiviral therapy against ZIKV infections is lacking. There is thus an urgent need to develop preventive and counteractive measures against this flavivirus. We initiated a high-throughput screening effort to identify novel and potent Zika antivirals that preferably also show broad-spectrum activity against other flaviviruses (e.g. dengue virus, yellow fever virus). Screening of two compound libraries, each consisting of ~300,000 compounds, lead to the identification of ~30 potential hit compounds. Follow-up of these potential hit compounds is currently ongoing. Novel ZIKV inhibitors should ultimately generate a positive Proof-of-concept (POC) in a ZIKV mouse model. We showed earlier that the nucleoside analog 7-deaza-2'-*C*-methyladenosine (7DMA) shows good efficacy in our ZIKV mouse model[2]. In view of preventing sexual transmission, novel inhibitors should preferably also be efficacious in lowering ZIKV titers in testis and seminal fluid, for which our ZIKV mouse model can also be employed. Indeed, preliminary data show that the 'tool' compound 7DMA significantly lowers viral RNA in the testis and epididymis of ZIKV-infected mice. We thus propose that novel ZIKV inhibitors should preferably also elicit activity in this model.

106. Nanoviricides Prevent Varicella Zoster Virus Infection in Human Skin

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Virucidal therapy reduces contagiousness, remains effective against mutants, and has possible broad-spectrum use. NanoViricides, Inc. has a platform to construct nanomicelles from a backbone of polyethylene glycol and alkyl pendants. Virus-specific ligands, designed using molecular modeling to mimic the binding site of virus on its cell surface receptor(s), are attached covalently to the backbone. Such nanoviricides™ have been constructed for herpesviruses, influenza virus, HIV, flaviviruses, and other emerging and bioterror agents. Polyvalent, cooperative binding of the ligands to the virion surface causes the "nanoviricide™" to engulf the particle, neutralizing and even dismantling it. Nanoviricides™ are effective against HSV-1 skin infection in mice, but they had not been tested against the related varicella zoster virus (VZV), which causes shingles. A panel of nanoviricides™ was evaluated in cell cultures, and some reduced VZV infectivity and cell-cell spread. These were evaluated in human skin organ culture compared to cidofovir as a positive control. Cell-free VZV-BAC-Luc strain was pre-incubated with nanoviricides™ for

1 h before inoculation into skin. Several reduced VZV infectivity on Day 1 and spread by Day 3-5. Nanoviricides™ were also applied to the skin 5 min after VZV inoculation, and several prevented infection. VZV infection was measured by bioluminescence imaging of the firefly luciferase reporter gene. Active compounds were formulated in different vehicles for topical application, which did not cause overt toxicity, as assessed by histopathology. Lead compounds are under selection for clinical trials for shingles topical therapy. The NanoViricides polymeric micelle technology is a promising approach for topical antiviral therapy.

107. Screening Campaign for the Identification of Novel Inhibitors of the Porcine Reproductive and Respiratory Syndrome Virus

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Porcine reproductive and respiratory syndrome virus (PRRSV) is a small enveloped single-stranded (+)RNA virus (genus *Arterivirus*, family *Arteriviridae*) consisting of two major genotypes. Both type 1 (European) and type 2 (North-American) are distributed globally and affect pigs at all stages of production. Infection results in respiratory disorders, early death of piglets and reproductive failure in sows leading to annual production losses >€2.0 billion. Current control measures include strict biosecurity measures, improved farm management and the use of killed or modified-live vaccines. Regardless, PRRSV is still present in most pig-producing areas due to its ability to mutate rapidly. Hence, antiviral inhibitors may provide a complementary control measure to combat the disease.

A screening campaign for the identification of novel PRRSV inhibitors was set-up and ~5120 compounds screened against the cytopathogenic PRRSV type 2 (VR-2332). A total of 74 out of the initial 268 hits (5.1% hit rate) displaying full inhibition of virus replication at 10 µM were confirmed using a cell-viability assay. Next, confirmed hits were tested against a non-cytopathogenic PRRSV type 1 (Lelystad) using RNA yield reduction assay. The identified pan-genotype hit compounds belong to several distinct chemical classes and are promising starting points for further hit-to-lead optimization towards the development of potent PRRSV inhibitors.

Potent, selective inhibitors will be characterized, their mode-of-action determined and eventually developed as fast-acting, pan-genotype anti-PRRSV drugs. Potential treatment options may include treatment of sows when entering a nursery facility and/or before gestation to clear the virus and minimize stillbirth and mummification of piglets.

108. Synthesis and Antiviral Eevaluation of Fluorescent T-1105 and T-1106 Analogues

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There is a great need for the development of drugs that have therapeutic efficacy in treating diseases caused by highly pathogenic RNA viruses. With the exception of ribavirin, only a few nucleoside analogues show broad-spectrum antiviral activity. Recently, the fluorinated pyrazinecarboxamide T-705 (favipiravir), and the defluoro analogues T-1105 and T-1106 have demonstrated their potency in treating viral infections in against several RNA viruses. With their unique mechanism of action and broad range of antiviral activity, these pyrazinecarboxamide derivatives are promising drug candidates and can therefore serve as a starting point for the development of new antiviral drugs.

Here, we would like to report on the chemical synthesis of fluorescent T-1105 and T-1106 analogues, as well as their antiviral evaluation against various DNA and RNA viruses. In addition, we investigated their fluorescent properties in different media, in order to use them as chemicals probes e.g. for cell uptake or cell extract hydrolysis studies.

109. Oral Combination Vaccine against Anthrax & Hepatitis B: Development and Characterization

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Infections are still 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 cost of immunization. Combination vaccines can answer these problems by development of single vaccine containing all possible antigens. The goal of present study was to see the effect of 2 antigens when given in combination. Bilosomes can provide needle free, painless approach for immunization. Recombinant hepatitis-B surface antigen(HBsAg) and recombinant protective antigen(rPA) were candidate antigens.

Bilosomes containing rPA and HBsAg were prepared by lipid cast film method. Antigen loaded bilosomes were characterized *in-vitro* for shape, size, antigen entrapment and stability in various body fluids. Fluorescence microscopy was done to confirm the uptake of bilosomes. The *in-vivo*study comprised of immunization of Balb/c mice and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. Bilosomes formed were multilamellar and stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken

up by gut associated lymphoid tissues. In-vivodata 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 and HBsAg given in combination, HBsAg(high dose) potentiates the production of anti-rPA antibody. Also they elicited measurable sIgA in mucosal secretions, while alum adsorbed antigens failed to elicit such responses. The combination produced both systemic as well as mucosal antibody responses upon oral administration.

110. Some Medicinal Plants from Nigerian Ethnomedicine Exhibited Antiviral Activity against Enteroviruses.

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Natural products provide a rich source for discovery and development of novel antiviral drugs due to available chemical diversity. Echovirus 7, 13, and 19 are part of the diseases causing enteric viruses identified from Nigeria. There is currently no antiviral drug nor powerful prophylaxis to employ against these enteric viruses.

Herein, by inhibition of viral cytopathic effect on rhabdomyosarcoma cells, we evaluated the antiviral activities of twenty seven (27) plant extracts from twenty six (26) different plants, obtained by random selection from ethnobotanical survey, against E7, E13, and E19. The maximum non-toxic concentration (MNTC) of each extract was serially diluted, and MTT colorimetric assay was used to evaluate the ability of the extracts to inhibit viral-induced cell death in tissue culture after 72 h. Statistical analysis was carried out using GraphPad prism.

Ten of the twenty seven extracts were found active. *Mondia whitei* leaf extract was the most active with an IC50 of 0.03843 µg/mL and 0.005354 µg/mL on E7 and E19, respectively. *M. whitei* also showed good selective index of 3447.83 and 24747.85 on E7 and E19, respectively. *Ipomea asarifolia* leaf extract also showed good activity against E7 (IC50= 0.00252 µg/mL).

E13 was resistant to all tested extracts. The antiviral activity of *I. asarifolia* on E7 was not detectable in two subsequent dilutions of the MNTC, which is very important in the determination of antiviral potential of a test agent. The active extracts could be good sources of selective antiviral agents to control infections due echovirus 7 and 19.

111. Synthesis and Anti-HIV Activities of CD4 Down-modulating, Pyridine-fused CADA Compounds

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Macrocyclic polyamine cyclotriazadisulfonamide (CADA) is a small molecule that down-modulates cell-surface expression of human cluster of differentiation 4 (hCD4). The mechanism of action involves binding to the signal peptide of hCD4, locking it in the channel across the membrane of the endoplasmic reticulum, and preventing its translocation into the ER lumen and subsequent transport of the mature protein to the cell membrane. Extensive structure-activity relationship studies have been done on CADA but the most potent analogs that have been synthesized do not have optimal drug-like properties, such as solubility and cell permeability. SH28 (IC_{50} 5.9 µM), the first pyridine-fused CADA analog, represents a novel molecular scaffold that may be promising for improving the potency and drug-like properties of CADA compounds, as pyridine is completely miscible with water. It is also mildly basic, so it should be protonated in the GI tract, but it can become neutral in cell membranes. Heterocycles, including pyridine, are common structural motifs in drugs that are currently on the market. Pyridine-substituted and further fused analogs of SH28 were synthesized following different synthetic routes. These compounds were tested for CD4 down-modulation and anti-HIV activities in cell cultures. Of the compounds tested, LAL018, a pyridine-fused CADA analog with a para-benzyloxy substituent, was found to be the most potent towards CD4 down-modulation, with an IC_{50} value of 0.42 µM in CHO. CD4-YFP cells. It was also tested for anti-HIV activity against three different HIV strains and was found to be the most potent anti-HIV compound having a pyridine-fused scaffold.

112. Prevalence of Hepatitis Infections in Type 1, Type 2 Diabetes and Latent Autoimmune Diabetes of Adults (LADA): Implications for Antiviral and Diabetes Treatments

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Diabetic complications may affect treatment success against HBV and HCV infections, but the associations between hepatitis infections and diabetic complications remain unclear. This study aims to assess hepatitis B and/or C infections in a large-scale population (n=1879) of type 1, type 2 diabetes mellitus (T1DM, T2DM) and LADA patients in China. HBV and HCV infections were detected using HBV preS1-Ag ELISA kits and HCV antibody ELISA kits, respectively. A total of T1DM (n=546), T2DM (n=996), LADA (n=337) patients was analyzed. Our results revealed HBV infections in 82 (15.0%) of T1DM patients, 133 (13.4%) of T2DM patients, and 40 (11.9%) of LADA patients. HCV infections were found in 9 (1.7%) of T1DM patients, 15 (1.5%) of T2DM patients, and 19 (5.6%) of LADA patients. Coinfections of HBV and HCV were observed in one T2DM and three LADA patients. Moreover, HBV prevalence (13.6%, 255/1879) in our diabetic patients was significantly higher than that in non-diabetic HBV patients (4.8%,1545/32150) collected by our meta-analysis ($p<0.0001$), but no difference of HCV prevalence between diabetic and non-diabetic patients was found (2.29% versus 2.18%, $p=0.76$). To discover host factors that drive the difference, we found aspartate transaminase (AST) and postprandial blood sugar(2PBS) were higher in diabetic HBV patients than that in non-diabetic HBV patients ($p<0.05$), while other factors showed no significance.
Our findings show HBV infections in diabetic patients are significant higher than non-diabetic patients, implying the importance of treatments against HBV and diabetes coinfections.

113. Antiviral Properties of Titanium Dioxide Nanoparticles against Human Adenovirus Serotype 5

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Development of nanotechnologies is related to the production of nanomaterials with novel properties and characteristics. Nanoparticles were shown to possess anti-bacterial, fungicidal and virucidal effects. For example, metal oxides nanopowders play an important role in the antiviral therapy. Use of these materials in water purification and elimination of waterborne pathogens is a developing procedure with multiple benefits. Adenoviruses is a group of viruses characterized by alimentary route of transmission. As a result, consumption of contaminated water is one of the possible ways of being infected with adenoviruses. The aim of the work was to analyze the ability of titanium dioxide nanoparticles to inactivate adenovirus serotype 5. It was shown that all experimental concentrations of nanoparticles did not have a toxic effect on cell culture. The analysis of virucidal action of nanoparticles showed that the highest activity (63%) was observed at low concentrations of nanoparticles. The antiviral activity of titanium dioxide nanoparticles against human adenovirus serotype 5 was within the range of 45-95%. As titanium oxide nanoparticles are characterized by photocatalytic properties, the antiviral effect of photoactivated titanium dioxide nanoparticles was also analyzed. It has been established that the virucidal action of nanoparticles increases by 30% when using ultraviolet irradiation.The obtained results confirm the promising use of titanium dioxide nanoparticles for the synthesis of inactivating compounds with disinfecting activity and their subsequent use as components of filters for water and air disinfection.

114. Anti-HIV Activity of Flavonoid Glycosides in Extracts from Deschampsia Caespitosa L. and Calamagrostis Epigeios L. (*In Vitro* and *In Silico* Study)

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BACKGROUND. The search for the most effective compositions is still a challenging issue in HIV treatment. Flavonoids of various groups have been already demonstrated to possess antiviral activities, in particular *in vitro* anti-HIV activity. The aim of the study was to assess specific anti-HIV activity of the extract from wild gramineous plants (Deschampsia caespitosa L., Calamagrostis epigeios L) and the flavonoid-enriched composition. The partially purified extract and composition (0.64 mg/ml calculated as rutin equivalent)) was used in anti-HIV assays. Anti-HIV activity of the composition was estimated by the reduction of the HIV infectious (BIII strain) titer in MT-4 cells and inhibition of reverse transcriptase activity. p24 expression was detected by EIA. 35 flavonoid derivatives representing the active structures of the herbal composition were taken from PubChem database. RESULTS. The crude flavonoid-containing extract exhibited substantial anti-HIV activity inhibiting the infectious titer of virus. IC50 - 0.18 μg/ml, IC90 - 0.52 μg/ml, flavonoid-enriched composition, IC50 - 0.08 μg/ml, IC90 - 0.32 μg/ml. SI of the composition - 412. Upon docking onto Nevirapine-binding site of the reverse transcriptase, all the putative variants of flavonoid derivatives including those verified as the ingredients of the composition have been shown to occupy this site with comparable efficacy. CONCLUSIONS. The flavonoid-containing composition possesses antiretroviral activity *in vitro* and *in silico*, as evidenced by significant reductions in HIV infectious titer and inhibiting reverse transcriptase. The further analysis of the possible cooperative interaction between various components of this herbal extract would be beneficial for designing the novel anti-HIV drugs.

115. Combination of Oseltamivir and a Novel Kinase Inhibitor Has Synergistic Antiviral Activity against Influenza Viruses

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The majority of approved antivirals target viral proteins. This makes them specific for a particular virus and susceptible to the development of resistance. For influenza, the only clinically useful drugs are the neuraminidase (NA) inhibitors. Resistance to oseltamivir can be conferred by single residue mutations in NA, many of which are present in seasonal strains of influenza virus. Similar mutations in NA could cause resistance to other FDA-approved NA inhibitors, highlighting the need for new influenza antivirals with a higher barrier to resistance.

The approach of this project is to develop antivirals that target a host factor and are not susceptible to resistance by viral mutations. From antiviral screening of a 900,000 compound library, M85 was identified as a high priority entry inhibitor of influenza A and B viruses with minimal toxicity. Preliminary studies suggest that M85 targets host kinases and inhibits endosome trafficking of influenza virus. Other experiments demonstrate the inability to select for M85 resistance mutations, underscoring the potential for M85 to be a novel pan-influenza virus inhibitor with a high barrier to resistance.

Since combining antivirals can boost their therapeutic effect, this study investigated the antiviral effects of combinations of M85 and oseltamivir. The combination index from an *in vitro* drug interaction assay indicated that they have strong synergism. *In vivo,* treatment with the combinations was more protective against a lethal viral challenge than oseltamivir alone. Overall, this combination could enhance treatment using NA inhibitors by increasing efficacy and reducing the susceptibility to resistance.

116. Effect of Double Antiviral Combinations Applied by Consecutive Alternating Administration against Coxsackievirus B1 Infection in Mice

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INTRODUCTION: Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with enormously high morbidity, including a series of severe illnesses that affect the CNS, heart, b-cells of pancreas, skeletal muscles, and so on. Unfortunately, there is no specific treatment or vaccine available for these infections, and the patients' treatment is mainly supportive. In the last few years our team has developed an experimental alternative treatment strategy based on consecutive alternating application (CAA) of inhibitors with different modes of action. This work represents the antiviral activity of double combinations of anti-enteroviral compounds applied via CAA course against the Coxsackievirus B1 neuroinfection in mice.

METHODS: Antiviral combination effects were examined by relying on double combinations of pleconaril, guanidine hydrochloride, MDL-860 and oxoglaucine put through CAA treatment scheme on CVB1 neuroinfection in ICR newborn mice infected s.c. with 20 MLD_{50}. Cumulative mortality (percentage), mean survival time (MST) (days) and weight (in grams) of suckling mice were recorded.

RESULTS: The CAA double combinations of pleconaril with MDL-860, guanidine hydrochloride and oxoglaucine demonstrated a marked activity *in vivo*. The highest effect was observed when pleconaril was combined with oxoglaucine (MST was increased 4.4 days more than placebo). The combinations of MDL-860 with oxoglaucine and guanidine hydrochloride on experimental CVB1 infection in mice were ineffective.

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117. Biophysical Analysis of Amphipathic α-Helical (AH) Peptides

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An antiviral strategy that directly targets viral lipid envelope has compelling features including broad-spectrum against enveloped viruses and suppression of emerging drug-resistant virus strains because viral lipid envelopes are derived from the host cell membranes. However, more in-depth mechanistic details how membrane-active agents interact remain elusive. Herein, we comparatively investigated two antiviral peptides (AH and C5A peptide) which are suspected to have a different mechanism of action against viral lipid envelope. We examined the biophysical characteristics of peptides including secondary structure change in membraneous environments by circular dichroism analysis, pore formation or lysis of tethered lipid bilayer by electrical impedance spectroscopy, and peptide-induced rupture of virus-mimicking tethered vesicles by epifluorescence microscopy with single-particle tracking analysis to capture the kinetics. We found that AH peptide exhibits low helicity of 30%, then increases to 65% upon the formation of a peptide-lipid complex by 1:10 ratio. Marked contrast, C5A reaches its maximum helicity of 80% from 1:4 of the peptide:lipid ratio. In tethered planar bilayer and single vesicle assay, C5A exhibited more aggressive and instantaneous interaction than AH towards lipid membranes after the peptide injection. Correlate with aforementioned finding, *in vitro* evaluation further proved that C5A has less antiviral therapeutic efficacy and more toxic to the human cell. Taken together, we suggest AH peptide scavenges lipids progressively forming α-helical structure to be stabilized whereas C5A causes a catastrophic effect on membrane similar to surfactant behavior. This finding may shed further insight into the design guidelines for antiviral peptides.

118. Evaluation of the Antiviral Activity of Anisomycin against Dengue and Zika Viruses: *In Vitro* and *In Vivo* Studies.

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We investigated the antiviral activity of the alkaloid anisomycin against dengue (DENV) and Zika (ZIKV) viruses. We first examined the effect of anisomycin on cell viability in Vero, A549, HepG2 and U937 cell lines. Non-cytotoxic concentrations of anisomycin caused a dose dependent inhibition of viral production in Vero cells, with 50% effective concentration values of 23.2, 31.3, 24.8, 61.6 and 33.0 nM for DENV-1, DENV-2, DENV-3, DENV-4 and ZIKV, respectively. Anisomycin exhibited antiviral activity in all the human cell lines tested and against DENV-2 clinical isolates. Inhibition of DENV-2 protein expression and viral RNA synthesis was demonstrated, but methyltransferase and RNA dependent RNA polymerase activities of DENV NS5 protein were not affected by the compound.

The toxicity and antiviral activities of anisomycin against ZIKV in a mouse model were also evaluated. AG129 mice were treated for 10 days with anisomycin (4, 20, or 100 mg/kg/d) beginning 4 h after ZIKV infection. A more rapid mortality rate was observed associated with treatment with 100 mg/kg/d in comparison to untreated infected mice, while animals treated with 4 mg/kg/d of anisomycin died significantly ($p<0.05$) later and presented lower viremia levels than the control group, indicating a reverse dose response effect of the compound.

In conclusion, anisomycin is a potent and selective *in vitro* inhibitor of DENV and ZIKV that impairs viral macromolecular synthesis, and treatment with a low dose of anisomycin (4 mg/kg/d) appeared to provide some minimal benefit in an AG129 mouse model. [Supported in part by HHSN272201000039I, Virology Branch, NIAID, NIH].

119. N-acylhydrazones as RNase H Selective Inhibitors Active against Replication of HIV-1 NNRTIs Resistant Variants

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HIV-1 infection requires a life-long treatment and faces the challenge of an increased rate of transmitted drug-resistant mutations with 2.1 million of new infections/year. Therefore, a constant and timely effort is needed to identify and develop new HIV inhibitors able to inhibit drug-resistant variants. Ribonuclease H (RNase H) activity of HIV-1 reverse transcriptase (RT) is a very promising target for drug development whose functional abrogation compromises the viral infectivity, but, up to date, it is the only viral enzymatic activity that still lacks an efficient inhibitor. We individuated an N-acylhydrazone derivative, compound **13**, able to inhibit viral replication (EC_{50} =10 μM), retaining full potency of inhibition against the NNRTI resistant double mutant K103N-Y181C virus (EC_{50} =12 μM) in an HIV-1 full-replication assay. Time-of-addition studies showed that compound **13** targets the reverse transcription step in cell-based assays. Biochemical assays showed that compound **13** inhibits the RT-associated RNase H function (IC_{50} =2.3 μM) being >20 fold less potent against the RT polymerase activity. Docking calculations reveal that compound **13** binds within the RNase H domain in an orientation different from the selective RNase H inhibitor RDS1759, and site-directed mutagenesis studies showed it is active against the Q475A RT, resistant to RDS1759, while it loses potency against the R557A RT that is fully responsive to RDS1759. Overall we have identified a novel compound that can be taken as starting point to generate a new series of more potent RNase H selective inhibitors active against circulating drug-resistant variants.

120. Mapping the Antiviral Chemical Space

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Large amounts of antiviral activity data for small molecule compounds are available in public repositories, such as ChEMBL and PubChem BioAssay. Methods of data mining have been developed rapidly during the last years, and they look attractive for large scale analysis and prediction of antiviral activity. We have developed a scheme of advanced curation for these data, including annotation of the target viruses according to the ICTV taxonomy and standardization of the representations of chemical structures.

Based on assay data available in ChEMBL, we compiled its subset, ViralChEMBL, containing approximately 620 thousands data points attributable to antiviral activity for more than 300 thousands compounds. Our data extraction approach, based on text search for virus-related substrings in assay descriptions, is three times more powerful than standard schemes. The web interface for this database is currently under construction.

For each compound an antiviral activity profile was generated, comprising information on viral species against which this compound is active or not. These profiles define the antiviral chemical space, the manifold of all available antiviral activity data. Chemical space mapping approaches were used to visualize this manifold and identify commercially available compounds that possessed the desired antiviral activity profiles against flaviviruses and enteroviruses. Experimental validation of the prediction confirmed its usability for prospective studies.

121. Activity of Double Combinations of New Diaryl Ethers against Coxsackievirus B1

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INTRODUCTION: Coxsackievirus (CV) infections have worldwide distribution and causes severe morbidity and mortality, particularly in the very young. Chemotherapy is an important tool for controlling CV infections, but clinically effective anti-enteroviral drugs do not currently exist, mainly due to the development of drug resistance and serotype diversity. In the last decades many molecules that selectively inhibit CV replication have been identified and characterized. We investigated the effects based on combination of inhibitors with different mode of action and some new synthesized diethyl ethers.

MATERIALS AND METHODS: Double combinations by newly synthesized diethyl ethers (derivatives of MDL-860) – CB-109, VGA-10-2 and VGA-12-2 with pleconaril, guanidine hydrochloride, and oxoglaucine were tested *in vitro* on HEp-2 cells for their activity against Coxsackievirus B1 strain Connecticut-5. Antiviral combination effects due to drug–drug interaction were examined by relying on the three-dimensional model developed by Prichard and Shipman (1990) by using the program MacSynergy™ II.

RESULTS: The combinations of CB-109 with pleconaril and oxoglaucine were synergistic with the exception of the additive effect with guanidine hydrochloride. The combination activities of VGA-10-2 with pleconaril, oxoglaucine and guanidine HCl were markedly synergistic. The highest volume of synergy is observed when VGA-10-2 was combined with pleconaril. The combinations including VGA-12-2 were mildly synergistic.

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122. *In Vitro* Antiviral Activity of λ Carrageenan against DENV Infection of Human Myeloid Cells in Absence or Presence of Antibodies

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Dengue is the most prevalent arthropode-borne viral human disease in tropical and subtropical regions, caused by four dengue virus (DENV) serotypes. In spite of the increasing global incidence, no specific antiviral therapy is available. Cells of the mononuclear phagocyte lineage are the main targets either for primary or secondary antibody (Ab)-mediated DENV infection, usually associated to severe forms of disease. Since virus entry may be a convenient therapeutic alternative, this study aimed to investigate the antiviral activity of ř carrageenan against DENV infection, in the presence and absence of Ab, in monocytic U937 cells and erythroleukemic K562 cells. The carrageenan was non cytotoxic for both human cells as determined by trypan blue exclusion method at concentrations up to 1 mg/ml. The antiviral effective concentration 50 % (EC50), measured by virus yield inhibition, was lower than 1.25 µg/ml against primary infection of both myeloid cells with all DENV serotypes. In Ab-mediated infection, the inhibitory activity was dependent on the cell and the Ab. In K562 cells, where immune complex entry is mediated through FcRII, the carrageenan was always inhibitory of Ab-mediated infection whereas in U937 cells, that express both FcRI and FcRII, there was inhibition only when FcRII was involved in Ab-dependent entry. The early events of virus adsorption and internalization were the targets of carrageenan in primary and Ab-mediated infection of myeloid cells. These results confirm that diverse factors of virus-host interaction should be considered for evaluation of safe entry inhibitors reactive against primary and secondary DENV infections.

123. Consecutive Alternating Treatment Strategy against Coxsackievirus B3 Infections

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Introduction: Coxsackieviruses (CVs) are relatively common viruses associated with a number of serious human diseases, including myocarditis and meningo-encephalitis. Many researchers have developed effective compounds against CVs – although no clinically available specific treatment currently exists, mainly due to the development of drug resistance. Our team developed the treatment strategy based on combination effects of enterovirus inhibitors in order to limit this process. Drug tests of the viral isolates showed that these drug combinations increase the viral sensitivity. In the current study, we define the virus isolates sensitivity to each compound included in the pleconaril/guanidine hydrochloride/oxoglaucine (PGO) and pleconaril/MDL-860/oxoglaucine (PMO) combinations applied via CAA treatment course.

Material and Methods: The antiviral activities of pleconaril, guanidine hydrochloride, MDL-860 and oxoglaucine on CVB3 cardiotropic (Woodruff) and neurotropic (Nancy) isolates from mice treatment via CAA course were determined through inhibitory concentration 50.

Results: The pleconaril sensitivity in the PMO/PGO via CAA course was preserved till the end of the Woodruff strain observation period. Viral isolates by Nancy strain were completely resistant to pleconaril in both PMO/PGO via CAA and monotherapy courses. Sensitivity to MDL-860 increased during CAA course with PMO vs. placebo and MDL-860 monotherapy. A minor increase of the susceptibility to guanidine hydrochloride during CAA course with PGO was observed. The sensitivity to oxoglaucine at PMO/PGO was also increased.

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124. Design, Synthesis and Evaluation of Novel Potential Antivirals Targeting the Norovirus Polymerase

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Norovirus is recognised as major cause of acute gastroenteritis in humans, affecting 684 million people every year and causing 218,000 deaths, with estimated annual costs of US $60 billion to the global society. There are currently no antiviral treatments available, making the development of novel agents for prophylaxis or treatment against norovirus an urgent need.

One of the most promising antiviral targets is represented by the viral RNA-dependent RNA polymerase (RdRp), which is the responsible for viral replication. A previous study in our research group aimed to identify novel non-nucleoside inhibitors of the norovirus polymerase through a virtual screening of commercial compounds, performed using two RdRp crystal structures (PDB ID: 3URO and 4LQ3). The 62 best compounds identified *in silico* were biologically evaluated and multiple hits were found to inhibit different calicivirus polymerases at low micromolar concentrations.

The present study aimed to explore various chemical modifications of the original hit compounds, in order to develop a series of novel small-molecule antiviral agents showing improved pharmacokinetic profiles and a greater ability to inhibit the norovirus polymerase.

Different chemical modifications on the original hit structures have been designed and several new derivatives have been synthesised. The synthetic strategies optimised to obtain the desired novel compounds, as well as their potential to inhibit the norovirus RdRp, will be discussed in this presentation.

125. Development of a Highly Efficient Bioreactor for Production of Chicken Egg Yolk Antibodies (Igy) as a Prophylactic and Therapeutic Agent for Human Norovirus

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Human norovirus (HuNoV) is responsible for more than 95% of outbreaks of acute non-bacterial gastroenteritis worldwide. Despite major efforts, there are no vaccines or effective therapeutic interventions against this virus due to the lack of an efficient cell culture system and a small animal model. Chicken immunoglobulin Y (IgY)-based passive immunization has been shown to be an effective strategy to prevent and treat many enteric viral diseases such as rotavirus. Here, we developed an efficient bioreactor to generate a high titer of HuNoV-specific IgY in chicken yolks using a recombinant vesicular stomatitis virus expressing HuNoV capsid protein (rVSV-VP1) as an antigen. We first demonstrated that HuNoV VP1 protein was highly expressed in chicken cells infected by rVSV-VP1. Subsequently, we found that White Leghorn hens immunized intramuscularly with rVSV-VP1 triggered a high level, long-lasting HuNoV-specific serum IgG and yolk IgY antibodies. The purified yolk IgY was efficiently recognized by HuNoV virus-like particles (VLPs) in ELISA and Western blot analysis. Importantly, HuNoV-specific IgY efficiently blocked the binding of HuNoV and VLPs to all three types (A, B and O) of histo-blood group antigens (HBGA), the function receptors of HuNoV in a saliva-based HBGA blocking assay. In addition, the receptor blocking activity of IgY remained stable at temperature below 70 °C and at pH ranging from 4 to 9. Thus, immunization of hens with VSV-VP1 could be a cost-effective and practical strategy for large-scale production of anti-HuNoV IgY antibodies for potential use as prophylactic and therapeutic treatment against HuNoV infection.

127. Family Outbreak of ECHO-3 Enterovirus Infection

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Non-Polio Enteroviral Infections (NPEVI) are emergent diseases presently.

AIM OF STUDY: to present results of the clinical and laboratory examination of family outbreak of NPEVI among children.

MATERIALS AND METHODS: The study group included 6 children who had close family contacts in August-October 2017. The middle age - 18 + 4.3 months. There were 3 boys (33%). NPEVI was confirmed by detection of RNA EV by PCR ("EV-PCR", Belarus) in samples of feces, blood and cerebrospinal fluid. The VP1 gene fragment of the capsid protein of EV has been used for molecular typing of isolated samples.

RESULTS: Family outbreak was characterized by a wide variety of clinical manifestations of laboratory-confirmed NPEVI: severe aseptic meningoencephalitis (AME) with persistent convulsive syndrome and impairment of consciousness - 1 case (16.6%), acute intestinal infection - 2 cases (33.3%), acute respiratory infection - 3 cases (50%). Based on the results of the molecular typing of enteroviruses in the feces of 3 out of 6 examined children, the ECHO-3 virus was identified. All samples of clinical material were negative for herpes simplex virus, human herpesvirus type 6, Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus.

CONCLUSIONS: A wide range of clinical manifestations of NPEVI in the family outbreak from a mild diseases to the development of severe AME has been presented, which indicates the complexity of the clinical verification of this infection and dictates the need for laboratory examination with modern molecular genetic methods to establish real of NPEV distribution in regions of Belarus.

128. The Ebolavirus VP35 Oligomerization Domain: Crystal Structures and Biophysical Characterization of a New Potential Antiviral Target

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The multifunctional protein VP35 encoded by ebolaviruses is a determinant of virulence and pathogenesis indispensable for viral replication and host innate immune evasion. Essential to VP35 function is the ability to undergo homo-oligomerization through self-assembly of a predicted coiled-coil motif. We report the crystal structures of VP35

oligomerization domains from the prototypic Ebola virus (EBOV) and the non-pathogenic Reston virus (RESTV), together with comparative biophysical characterization of the domain from all other known species within the *Ebolavirus* genus (Sudan, Taï Forest and Bundibugyo viruses). We show that trimeric EBOV and tetrameric RESTV VP35 oligomerization domains have bipartite parallel helix-bundle structures with a canonical coiled-coil in the N-terminal half and increased plasticity in the highly conserved C-terminal half. Size exclusion chromatography coupled with multi-angle light scattering and native mass spectrometry demonstrate that EBOV VP35 oligomerization domain assembles into trimers and tetramers, while in RESTV and other ebolaviruses it exclusively forms tetramers. Circular dichroism and differential scanning fluorimetry indicate that all ebolaviral VP35 oligomerization domains have a similar coiled-coil folding - which allowed homology modeling of the EBOV VP35 oligomerization domain tetramer - but vary in secondary structure content and thermal stability. Site-directed mutagenesis shows that substitution of leucine residues in the coiled-coil, which were previously found critical for replication and innate immune antagonism, causes aberrant oligomerization. Newly-identified mutations demonstrate that a conserved arginine involved in inter-chain salt-bridges stabilizes the coiled-coil and modulates between VP35 oligomeric states. These findings provide a framework for rational, structure-based therapeutics design on a potential antiviral target.

129. Dual Effect of the Multi-Kinase Inhibitor Midostaurin on Acute and Latent HIV-1 Infection

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In an effort to identify novel HIV-1 latency reversing agents (LRAs), we evaluated the effect of midostaurin in HIV-1 infection. Midostaurin is a multi-protein kinase inhibitor (including cyclin-dependent kinases, CDKs), that is used for the treatment of acute myeloid leukemia (AML). Midostaurin blocked HIV-1 replication in acutely infected cells at concentrations that were not cytotoxic. The antiviral effect was dependent on the expression of SAMHD1, a virus restriction factor which is, in turn, dependent on CDKs activity. The antiviral effect was also dependent on constitutive activation of FTL3 (CD135), the pharmacological target of midostaurin in AML. Following SAMHD1 degradation or wild type FTL3 expression the antiviral effect was lost and increased HIV-1 replication was observed as compared to untreated cells. In HIV-1 latently infected lymphoid cells, including clonal (ACH-2) and non-clonal models (J-HIG), midostaurin significantly increased HIV-1 reactivation. Moreover, a synergistic effect was observed when midostaurin was combined with known latency reversing agents (LRA), the HDAC inhibitors panobinostat and vorinostat, suggesting a distinct mechanism of action than the HDAC inhibitors.

In conclusion, we show a dual effect of midostaurin by blocking early steps of virus replication in acutely infected cells but promoting reactivation in latently infected cells. Our results suggest that agents such as midostaurin could effectively limit the size of the HIV-1 reservoir while preventing subsequent rounds of infection.

130. Development of a "Hepatitis B Virus (HBV) Persistence" Mouse Model Amenable for Antiviral and Vaccine Evaluation

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Approximately 257 million people worldwide chronically infected with HBV are at increased risk of developing hepatocellular carcinoma (HCC) and associated liver complications. Despite the availability of potent nucleoside/nucleotide inhibitors such as Entecavir and Tenofovir, there are no curative therapies available. HBV cure research is hampered by the lack of a suitable small animal (mouse) model that can recapitulate HBV persistence and facilitate the evaluation of antivirals and therapeutic vaccines.

To address this need, an immunocompetent mouse model for HBV persistence was developed using an adeno-associated virus expressing the HBV (AAV-HBV). Mice showed dose dependent serum HBs Antigen (Ag) and HBV DNA levels. HBs Ag expression was observed up to 17-weeks.

Entecavir was tested as an antiviral prophylactically decreased in serum HBV DNA at 15 days post-treatment. The Engerix-B vaccine used as a vaccine therapeutically, cleared serum HBs Ag and induced HBs antibody response. Additional characterization of cccDNA levels and RNA profiling in these mice is currently ongoing.

The AAV-HBV model offers important advantages to currently available alternatives. It does not require engraftment and it is cost-effective (compared to human liver chimeric mouse). Suitability of testing vaccines and antivirals (data presented in this abstract) along with the previous report of fibrotic disease phenotype suggests that AAV-HBV model could be an attractive tool for testing the proof of concept (POC) of novel antivirals and vaccines to further advance HBV cure research.

131. Development and Characterization of an Aerodynamic System for Pulmonary Delivery of Influenza Vaccine

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The name *influenza* is Italian and means "influence", Commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family Orthomyxoviridae , that affects birds and mammals.The aim is also to develop and characterize aerodynamic systems with r-H1N1Ags for safely deposition in alveoli to enhance the bioavailability and control release of influenza antigen after pulmonary administration in animal model. This Induces not only systemic humoral (IgG) responses, but also cell-mediated (IL-4, IFN-ř) and mucosal immune responses (IgA, IgG), non-invasive, propellant & needle free delivery of vaccine.

The chitosan microparticles were prepared by ionic gelation method of chitosan with tripolyphosphate(TPP). The formulations were optimized on the basis of particle size, tap density & entrapment efficiency. The external morphology of the optimized formulation was studied by TEM & SEM.The zeta potential was determined along with stability studies at accelerated tempreatures. The in-vivo studies involved determination of antibody titres in serum and mucosal secretions and uptake studies by fluorescence microscopy.

The results show that as the preparation was reduces to lyophilized form which increased the stability as compared to conventional liquid formulations.The microparticles of uniform size distribution were obtained owing to the repulsion between the positively charged particles. The fluorescence images show the uptake of microparticles by various organs and the ELISA results shows comparable IgG responses along with IgA.

Thus, Antibody production was found to be more in pulmonary route as compared to other routes.

132. Exploring Strategies to Improve Therapeutic Efficacy of Antiviral Drugs Targeting the N-linked Glycan Processing Pathway for Treatment of Viral Hemorrhagic Fevers

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Several genome-wide screening efforts identified ER oligosaccharyltransferase and glucosidases in the protein glycosylation pathway as host dependent factors of flaviviruses. Pharmacologically, ER glucosidases are among the few host proteins being validated *in vivo* as broad-spectrum antiviral targets. Two iminosugar based glucosidase inhibitors, UV-4 and Celgosivir, are currently being evaluated in clinical trials to treat dengue patients. We report herein the continuing preclinical development of an ureido deoxynojirimycin glucosidase inhibitor, IHVR-19029. IHVR-19029 is the only iminosugar reported to have activities *in vitro* against one or multiple members of each of the four families of viruses causing hemorrhagic fever, as well as *in vivo* activities against Ebola and Marburg viruses in mouse models. However, oral administration of 19029 was limited by poor bioavailability and GI distress due to off-target inhibition of GI-resident glucosidases. We therefore designed a serial of oral available prodrugs and identified a dibutyrate prodrug, 19029-2B, which showed overall higher active drug exposure and improved tolerability in mice, as well as improved *in vitro* and *in vivo* efficacy against Ebola virus in a lethal mouse model. Interestingly, only 1-2 logs reduction of flaviviruses were achieved in glucosidase knockout cells, suggesting that a limited extent of antiviral potency can be expected by complete target inhibition. We thus also explored the possibility to improve the antiviral efficacy of IHVR-19029 *via* combination therapy with other broad spectrum antiviral drugs and demonstrated that combination of sub-optimal doses of IHVR-19029 and T-705 significantly increased the survival rate in a mouse model of Ebola virus infection.

133. *Cuscuta reflexa* (Giant dodder): Potential Inhibitor of HCV NS3 Serine Protease

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HCV, a silent predator associated with 350,000 deaths in 2017, affects more than millions globally each year and remains veiled till the appalling symptoms like serious liver scars and hepatic cancer surge. NS3 serine protease (SP) is an indispensible enzyme that enhance the HCV pathogenesis by down regulate the host intrinsic immune responses and considered the most striking target for development of anti HCV therapy. The present work was intended to explore the anti-HCV potential of Giant dodder (*Cuscuta reflexa)* seeds extracts specifically targeting their NS3 gene.

C. reflexa, a renowned medicinal plant of South Asian conventional medicinal system, has been reported frequently for its broad spectrum pharmacological aspects. Trypan blue dye exclusion method and MTT assay were used to determine cellular toxicity effects of all phytoextracts on viability of Huh-7 cells. Then antiviral potential of extracts was assessed against HCV NS3-SP by transfecting HCV NS3 protease plasmid into liver cells. The plant organic extract was screened for the presence of phytochemical through standard procedures. The results revealed that non toxic methanolic extract of Giant dodder (*Cuscuta reflexa)* reduced the HCV NS3 protease expression (78%) in a dose-dependent manner and GAPDH remained constant. Four fractions were obtained through bioassay guided extraction and the ethyl acetate fraction exhibited maximum inhibition of NS3 enzyme. These results suggest that Dodder extract hold some leading natural chemical entities as lead compounds that can be potential agents against HCV. The combination of extract with interferon could also offer a future option to treat chronic HCV.

134. Successful Treatment of Ebola Virus Infected Nonhuman Primates with Hyperimmune Equine Immunoglobulin Fragments

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Ebola virus causes a severe haemorrhagic fever in humans with a case fatality rate from 50-90%. The 2013-16 Ebola outbreak in West Africa has claimed >28,000 infections and 11,300 deaths. Clinically approved countermeasures are urgently needed. Monoclonal antibody (mAb)-based candidates, such as ZMapp and MIL-77, are especially promising treatments due to their ability to reverse advanced EBOV disease in humans and nonhuman primates (NHPs), but can be expensive and time-consuming to manufacture in large quantities, thus restricting their usefulness in areas with limited resources. Our previous work showed that hyperimmune equine immunoglobulin fragments F(ab')2 strongly neutralize EBOV infection *in vitro*, and completely protect mice and guinea pigs against EBOV infection *in vivo*. Our aim is to characterize the protective efficacy of equine F(ab')2 in NHPs. Our results showed that administration of equine F(ab')2 initiated at 3 dpi resulted in 100% protection and the treated animals showed virtually no observable signs of disease throughout the experimental course. Measureable parameters indicate that the animals remained healthy and did not shed virus, which gave them time to develop a robust immune response against the infection. Treatment started at 5dpi also achieved 100% protection although all 3 animals showed signs of disease and recovered eventually after full treatment course. Our data indicates that Equine F(ab')2 is effective as a post-exposure treatment in NHPs. Due to well-established production protocols and good safety record, equine F(ab')2 should be considered as an alternative to mAbs for large-scale treatment of patients in the event of another EBOV epidemic.

135. A-to-I Editing by ADAR1 Limits HPV Expression by Regulating Innate Immunity

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Infection by human papillomavirus (HPV) alters the microenvironment of keratinocytes as a mechanism to evade the immune system. A putative therapeutic strategy against HPV infection would be the induction of an anti-inflammatory state to avoid immune response. Therefore, understanding the role and function of innate immune effectors and modulators can help to establish novel strategies for HPV treatment. A-to-I editing by ADAR1 has been reported as a key step in triggering innate immunity in response to foreign viral RNAs. We used human keratinocyte HaCaT(HPV-) and SiHa(HPV16+) cell lines to characterize innate immune activation in the context of HPV infection. SiHa HPV16+ cells showed lower expression of ADAR1, and higher expression of RIG-I and phosphorylated STAT1 compared to HaCaT HPV- cells. Thus, RNAi was used to specifically downregulate *ADAR1* for further characterization. *ADAR1* knockdown (siADAR1) in HPV16+ cells induced increased expression of *IFIH1*/MDA5 (24-fold), *DDX58*/RIG-I (20-fold), *IRF7* (13-fold), *IFNB1* (100-fold) and *CXCL10* (490-fold) compared to mock-transfected cells. Importantly, siADAR1 significantly enhanced *HPV16* expression, indicating ADAR1 and its downstream effectors may modulate HPV infection by innate immune activation. Functionality of ADAR1 and its ability to edit HPV transcripts was evaluated. A-to-I editing was found in known cellular target of ADAR1 (*NEIL1*), but was not identified in HPV transcripts, suggesting the effect of ADAR1 on HPV is editing-independent. In summary, we demonstrate that ADAR1 and its downstream effectors have an antiviral role in HPV infection through the induction of innate immunity. Hence, targeting ADAR1 could be a strategy against HPV infection and disease.

136. NonInvasive 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 concern about new virulent disease outbreaks. Immunization is a prophylactic approach through which body is shielded from any incoming pathogenic invasion. Immune response elicited by Topical Immunization depends upon structure and composition of 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 coadministered antigen and moreover it avoids first pass phenomenon and also protects antigen from enzymes that are present in gut wall.

Niosomes are nonionic surfactant based vesicles that were used as topical carrier for immunogens for transdermal delivery. The goal of the present study was to investigate potential of niosomes as carrier for HepatitisB antigen(HbsAg) with cholera toxin(CTB) as adjuvant. Niosomes prepared by Sonication. Antigen loaded Niosomes were characterized *invitro* for their shape, size, %antigen entrapment and stability. *Confocal laser scanning microscopy(CLSM)* was carried out to confirm uptake of Niosomes. The *invivo* part of study comprised of immunization of Balb/c mice and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Niosomes formed were multilamellar and stable. Presence of fluorescence at different skin depths reflected accumulation of these niosomes in the region of epidermis, suggesting better uptake of antigen by langerhans cells. Based on the results obtained, niosomes presented its potential for antigen delivery through transcutaneous route.

137. Characterization of Proteases of a Clade-D Betacoronavirus

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The outbreak of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002/2003 caused severe pneumonia in about 8100 cases, with a case/fatality rate of 10%. Before this epidemic, coronaviruses were considered harmless for humans, causing only non-symptomatic or mild infections. Derived from one or more bat coronaviruses, SARS-CoV impressively demonstrated the pathogenic potential of zoonotic CoVs. This conclusion is validated by the ongoing outbreak of Middle-East Respiratory Syndrome coronavirus (MERS-CoV), with 2143 reported cases since 2012 and a fatality of 35%. Both viruses belong to the genus Betacoronaviruses but are placed in different clades (B and C, respectively). Clade D of the betacoronaviruses comprises only one member so far, the relatively poorly characterized bat-CoV HKU9. In an effort to increase preparedness against future zoonotic CoV outbreaks, we focus on the characterization of the two HKU9 proteases, the papain-like protease (PLpro) and the main protease (Mpro or 3CLpro) as drug targets. Here we present the crystal structure of the HKU9 PLpro at 2.7 Å resolution and discuss similarities and differences from other CoV PLpros, in particular with respect to zinc-binding.

138. Development of Cholesterol-conjugated Stapled Peptides as Inhibitors of EBOLA Virus Infection

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The Filoviridae family consists of five ebolavirus species, Ebola (EBOV), Sudan (SUDV), Bundibugyo virus (BDBV), Reston (RESTV), and Taï Forest (TAFV) viruses, and a Marburgvirus species, with Marburg (MARV) and Ravn (RAVV) viruses. Ebola virus (EBOV) has emerged as a significant public health concerns since the 2013-2016 Ebola Virus Disease outbreak in Western Africa. Currently, there are no therapeutics approved and the need for Ebola-specific therapeutics remains a gap. In search for anti-Ebola therapies we tested the idea of using inhibitory properties of peptides corresponding to the C-terminal heptad-repeat (HR2) domains of class I fusion proteins against EBOV infection. The fusion protein GP2 of EBOV belongs to class I suggesting that a similar strategy to HIV may be applied to inhibit EBOV infection. The serum half-life of peptides were expanded by cholesterol conjugation to allow daily dosing. The peptides were further constraint to stabilize a helical structure to increase the potency of inhibition. The IC_{50} of lead peptides were in low micro-molar range, as determined by high-content imaging test of EBOV infected cells. Lead peptides were tested in a EBOV lethal mouse model and efficacy of the peptides were determined in 14 days study with BID administration of peptides for 9 days. The most potent peptide was able to protect mice from lethal challenge of mouse adapted Ebola virus. These data show that engineered peptides coupled with cholesterol can inhibit viral production, protect mice against EBOV infection, and may be used to build novel therapeutics against EBOV.

139. Hijacking of PI4KB by Picornaviruses – Structural Insights and the Role of ACBD3

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Picornaviruses are small positive-sense single-stranded RNA viruses that include many important human pathogens. Within the host cell, they replicate at specific replication sites called replication organelles. To create this membrane platform, they hijack several host factors, most importantly the lipid kinase PI4KB (phosphatidylinositol 4-kinase B). Many viruses including the Aichi virus, Coxsackievirus B3 or Enterovirus 71 use the Golgi resident acyl-CoA-binding domain-containing protein-3 (ACBD3) to hijack PI4KB. Recently, we structurally characterized the interaction of ACBD3 and PI4KB and we solved the structure of the 3A:ACBD3 protein complex. Our structural analysis reveals that the viral 3A proteins act as molecular harnesses to enslave the ACBD3 protein leading to its stabilization at target membranes and to PI4KB recruitment. Our data provide a structural rationale for understanding how these viral-host protein complexes assemble at the atomic level and identify new potential targets for antiviral therapies. Furthermore, because the 3A protein was from the Aichi virus, Coxsackievirus B3 or Enterovirus 71 our structural analysis reveals convergent evolution of ACBD3 and PI4KB hijacking among different groups of picornaviruses.

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140. Phenotypic Screening Funnel for Evaluation of Therapies against BSL-3 Neurotropic Alphaviruses

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The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) Therapeutics Development Center (TDC) developed a high throughput, image-based phenotypic screening process to characterize host-viral interactions in Biosafety Level (BSL) 2 to 4 laboratories. Our platform provides the ability to conduct combinatorial studies to evaluate multiple phenotypic signatures (e.g. antiviral, toxicity, anti-proliferative) critical to anti-viral therapeutic design.

The real power of phenotypic screening resides in the disease-relevance of the *in vitro* models chosen for the screening funnel. All three arboviruses are known to infect both neuronal cells and astrocytes. The development of the Stemonix® microBrain® Assay Ready platform, 384-well plates with a fully functional mixture of differentiated cortical neurons and astrocytes, allowed us to simultaneously evaluate the potency of anti-viral therapies against neurotropic viruses such as alpha viruses (eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV), and Venezuelan encephalitis virus (VEEV) in both neurons and astrocytes.

In this work, we show the development of a phenotypic screening funnel to provide an efficient primary filter for down selection of potent molecules for further evaluation in PK/PD and Tox models and/or efficacy in rodent models.

141. Nano-delivery for Drugs with Antiviral Properties Based on BSA as a Carrier

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The emergence of antiviral drug-resistant mutants is the most important issue in current antiviral therapy. An ideal therapeutic target to prevent drug-resistance development is represented by host factors that are crucial for the viral life cycle. Given that viruses are obligate parasites, several host factors that are crucial for viral replication also represent antiviral therapeutic targets, referred to as the "cell-based approach".

Recent efforts have focused on finding and characterizing cellular metabolic inhibitors as broad-spectrum antivirals for targeting with minimum cytotoxicity. MPA is a non-nucleoside, non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). It is well known by its effects as immunosuppressive and against multiplication of several viruses; this inhibition was highly reversed by exogenous guanosine addition, indicating that antiviral activity is effectively associated with GTP depletion through IMPDH blockade.

Albumin is emerging as a versatile protein carrier for drug targeting and for improving the pharmacokinetic profile of peptide or protein-based drugs. Principally, three drug delivery technologies can be distinguished: coupling of low-molecular weight drugs to exogenous or endogenous albumin, conjugation with bioactive proteins and encapsulation of drugs into albumin nanoparticles.

Using the self-assembly and salvation methods we obtained nanoparticles of approximately 10 and 100-200 nm respectively, determinated by scanning electron microscopy and Zeta-sizer Nano-Zs particle analyzer. The particles were evaluated for their cytotoxic effect in different cell lines cultures proving to be nontoxic, according to MTT method using standard concentrations. The preliminary results showed that this type of carriers based on albumin may be used for the antiviral drug delivery.

142. Screening of an FDA-approved Compound Library Targeting the mRNA Capping of Venezuelan Equine Encephalitis Virus (VEEV)

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Venezuelan Equine Encephalitis Virus (VEEV), an important re-emerging alphavirus from the *Alphavirus* genus, is a significant human and equine pathogen. Alphaviruses have a unique viral mRNA capping mechanism catalysed by the non-structural protein 1 (nsP1). Thus, nsP1 is an attractive target for drug design. First, the S-Adenosylmethionine (AdoMet)-dependent methyltransferase (MTase) activity of nsP1 methylates the N7 position of a GTP molecule to form ^{m7}GTP. Then, the enzyme catalyses the formation of a covalent bond between a conserved histidine and ^{m7}GTP leading to the formation of the ^{m7}GMP-nsP1 complex (guanylylation, GT). The last step is the transfer of the ^{m7}GMP from nsP1 to the 5′ end of the diphosphate viral mRNA forming the cap-0 structure, which is essential for RNA translation into viral proteins.

We previously demonstrated that the nsP1 guanylylation can be monitored by Western-blot using an anti-cap antibody. Using this detection strategy, we developed an ELISA assay to quantify the ^{m7}GMP-nsP1 adduct and identify new nsP1 inhibitors by screening 1220 FDA-approved compounds from the Prestwick Chemical libraries. We selected hits showing more than 80% inhibition effect at 50µM concentration. The hits were then confirmed by IC_{50} determination, an orthogonal assay on the nsP1 MTase activity, and specificity against a cellular MTase. Compared to the capping inhibitor Sinefungin, the best hits showed higher inhibition potency and nsP1 specificity. Altogether, the results show that this enzyme-based screening is a convenient way to select potent compounds targeting the mRNA capping of Alphaviruses and provide initial hits for the development of possible antivirals.

144. Acetohydroxamic Metal-Chelators against Hepatitis C Virus and Flaviviruses

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Hepatitis C Virus (HCV) infections pose a major public health threat globally, with infected individuals being at risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. There is no vaccine available and despite advances in current chemotherapy, the global burden of HCV infections remains high, due to their partial effectiveness or resistance. The flaviviruses Dengue (DENV), Yellow fever (YFV), and Zika (ZIKV) cause diseases ranging from mild febrile illness to severe encephalitis or hemorrhagic syndromes. Despite the extensive research on flaviviral diseases, there is no clinically approved therapy, thus, they constitute high priority targets for drug discovery.

Because of all the above and based on literature reports on metal-chelating agents inhibiting HCV NS5B-polymerase, the development of novel scaffolds of broadly effective metal-chelators with antiviral properties was undertaken. By utilizing docking-scoring calculations, structural insight regarding HCV inhibition was obtained, prompting the rational design and synthesis of novel carbocyclic-substituted hydantoin-derivatives, bearing the acetohydroxamic acid metal-chelating group upon the imidic nitrogen, and a variety of lipophilic substitutions at the amidic nitrogen. All the compounds were fully characterized and evaluated for their effect on HCV RNA replication and cell viability.

As flaviviruses are members of the Flaviviridae family, along with HCV, the synthesized compounds were additionally evaluated against DENV, YFV and ZIKV. Biological results, along with theoretical simulations, suggest that the novel class of the metal-chelators, presented herein, offers a highly promising starting point for the design of potent and specific antiviral agents.

145. Inhibition of Coronaviruses by Beta-D-N4-hydroxycytidine (NHC)

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The recent emergence of very virulent epidemic coronaviruses (CoVs), such as severe acute respiratory syndrome (SARS)-CoV and Middle East respiratory syndrome (MERS-CoV)-CoV, emphasizes the need for broadly active antivirals that inhibit a range of CoVs. There are currently no approved antivirals or vaccines for the treatment and prevention of CoV infections. Development of nucleoside-based antivirals for CoV infections has been hampered by the presence of a viral proofreading 3′-5′ exoribonuclease (ExoN). ř-D-N^4-hydroxycytidine (NHC), also known as EIDD-1931 (Emory Institute for Drug Development), has been reported to inhibit multiple viruses, including several alphaviruses, Ebola virus, and hepatitis C virus. Here, we report that NHC inhibits CoVs, including murine hepatitis virus (MHV) with a 50% effective concentration (EC_{50}) value of 0.17 mM and no cytotoxicity across a wide range of concentrations. Addition of exogenous cytidine and uridine restored viral replication in the presence of NHC, suggesting that NHC competes with natural pyrimidine nucleotides for incorporation into CoV RNAs. To specifically address the role of ExoN in CoV inhibition by NHC, we tested a proofreading-deficient mutant [ExoN(-)] of MHV. ExoN(-) virus exhibited increased sensitivity to NHC compared to wild-type virus. both by virus titer reduction and EC_{50}. Together, these results support further pre-clinical development of NHC for treatment of CoV infections as well as investigations into the mechanism by which NHC inhibits CoV replication.

146. SAMHD1 is a Modulator of Nucleos(t)ide Analogues Efficacy

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Nucleos(t)ide analogues, are commonly used in the treatment of infectious disease and cancer. SAMHD1 is a dNTP triphosphohydrolase involved in the regulation of intracellular dNTP pool, whose function has been linked to viral restriction, cancer development and autoimmune disorders. Here, we evaluate SAMHD1 function on antiviral and antiproliferative efficacy of a wide range of nucleos(t)ide analogues currently used to treat infections and cancer. Anti-HIV-1 and cytotoxic activity of compounds was assessed in primary and established cell lines in the presence or absence of SAMHD1. SAMHD1 effectively modified the anti-HIV-1 activity of all nucleos(t)ide analogues tested, whereas sensitivity to a non-nucleoside inhibitor (nevirapine) or to nucleoside phosphonates (cidofovir and tenofovir) was not effected. Interestingly, SAMHD1 could either enhance (gemcitabine, capecitabine, fluorouracil and floxuridine) or inhibit (Ara-C, fludarabine, cladribine, clofarabine and nelarabine) antiviral potency of anti-cancer analogues, an effect that was not dependent on the specific nucleotide targeted. When cytotoxicity was evaluated, SAMHD1-dependent changes were less evident and restricted to the increased efficacy of fluorouracil and floxuridine and reduced efficacy of nelarabine and ara-C in the presence of SAMHD1. In summary, our results demonstrate that SAMHD1 modifies the efficacy of a wide variety of nucleoside analogues used to treat infections, cancer and other diseases. In addition, anti-HIV activity of nucleos(t)ide analogues may represent a more sensitive measure of SAMHD1 impact on drug efficacy. Thus, modulation of SAMHD1 function may constitute a promising target for the improvement of multiple therapies.

147. Low Fidelity of SARS-Coronaviruses Polymerase Complex

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Coronaviruses infect a broad range of humans and animals and are the most common cause of respiratory and enteric diseases. An outbreak of SARS-CoV in 2003 and a recent outbreak of MERS-CoV in 2012 with high mortality rates have confirmed that these viruses are the potential risk for world public health. They follow a complex replication and transcription mechanism to replicate the largest RNA genome (27-32kb) of all known RNA viruses. We had previously demonstrated that nsp12 RNA-dependent RNA polymerase (RdRp) requires co-factors nsp7-nsp8 to be activated and confer replication processivity. Likewise, SARS-CoV uses a unique RNA proofreading mechanism involving the RdRp nsp12 and the ExoN nsp14 that had never been described in any RNA virus before. By using biochemical assays, we characterize here the fidelity of nucleotide incorporation by the polymerase complex nsp12/nsp7-nsp8 and compare it to other viral RdRps. Single nucleotide and mismatch incorporation assays were performed using different templates

(40nt) annealed to a 5′ radiolabeled primer (20nt) with increasing concentrations of nucleotides. The result shows that, in the absence of the Exonuclease (ExoN) excision activity, the polymerase exhibits low fidelity of RNA synthesis. The active site structure was compared to higher fidelity polymerases, and Coronavirus-specific structural determinants proposed for this observation. Our work predicts that larger ExoN-containing RNA virus genomes are theoretically possible, and extends knowledge about RNA genome virus evolution.

148. Human Parainfluenza-3 Inhibition by Nucleoside Analogues in a Human Airway Epithelium Cell Model

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Human parainfluenza virus infections have been recognized as a significant cause of morbidity in patients with leukemia, patients after lung transplantation and hematopoietic stem cell transplantation. We report the screening of a nucleoside library for inhibitors of HPIV-3 and the use of human airway epithelium cells of bronchial origin (HuAEC) in an air-liquid interface to study HPIV-3 replication.

A library of ~400 nucleosides was tested *in vitro* on a HPIV-3 Strain JS / LLC-MK2 model. Only one nucleoside, 2′-amino-2′-deoxyadenosine, was found to have some selectivity (EC50 7 µM, CC50 = 50 µM) under the specific test conditions. This molecule has previously been shown to inhibit measles virus.

In addition, we studied the replication of HPIV-3 on HuAEC and the antiviral effect of nucleoside analogues. Apical infection of these cultures with HPIV-3 strain JS and with a clinical isolate of HPIV-3 showed significant viral replication. Within 72 hr. p.i. a culture of 0.5 cm^2 secretes from the apical site ~10^8 copies of vRNA every 24 h and this is maintained for >15 days. To explore the effect of antiviral treatment cells were exposed, at the basal site, to nucleosides (100 µM Ribavirin or 100 µM of Favipiravir) from day -2 until day 4 p.i. A clear inhibition (>4 log reduction of vRNA) of HPIV-3 replication in the presence of Ribavirin could be observed while there was no effect of Favipiravir. When Ribavirin treatment was stopped, PIV-3 replication increased in 48 h to the level of untreated cultures.

149. Negative Charge and Membrane Tethered Viral 3B Cooperate to Recruit Viral RNA-Dependent RNA Polymerase $3D^{pol}$.

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The replication of picornaviruses is mediated by viral RNA dependent RNA polymerase $3D^{pol}$. The $3D^{pol}$ replicates RNA at replication organelles (ROs) also known as replication factories that are derived from the host membranes. Lipid hallmark of the Golgi - the phosphatidylinositol 4-phosphate (PI4P) lipid - was implied in recruitment of $3D^{pol}$ to ROs. ROs provide the microenvironment needed for efficient viral replication and serve as a shelter from innate intracellular immunity. However, picornaviruses do not possess any phosphatidylinositol 4-kinase (PI4K), instead they hijack the human PI4KB or PI4KA. Many picornaviruses hijack the lipid kinase PI4KB using the Golgi resident acyl-CoA-binding domain-containing protein-3 (ACBD3), a binding partner of the viral protein 3A [1].

Here, we aimed to reconstitute hijacking of PI4KB by the nonstructural viral 3A protein via the ACBD3 protein using pure recombinant proteins and biomimetic membranes. Upon hijacking, PI4KB starts producing PI4P lipid which leads to hyperphosphorylated membranes. Using our system, we discovered that not PI4P but rather the negative charge is responsible for the recruitment of $3D^{pol}$ to the membrane because another negatively charged lipid (PS) had the same effect whereas neutral membranes failed to recruit $3D^{pol}$ [2]. Additionally, we showed that membrane tethered 3B protein cooperates with the negative charge to increase the efficiency of $3D^{pol}$ recruitment.

1. Klima, et al.(2017) K, *Structure.* 25, 219-230.
2. Dubankova, et al.(2017), *Scientific reports.* 7, 17309.

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150. Use of a Dual Luciferase Cell-Based Drug Screening Assay to Study VP24 Inhibition of JAK/STAT Pathway and its Reversion by Compounds

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Ebola virus has evolved several and diversified strategies to antagonize the interferon (IFN) response in target cells leading to a complete impairment of the innate immune system which contribute to the pathogenesis of infection. A decisive role is exerted by VP24 that disables cells to contrast viral replication and propagation by inhibiting the IFN system at level of JAK/STAT pathway. The early control of viremia is one of the keys for survival, hence, blocking an important determinant of virulence such as VP24 is a valuable subject for investigation and a crucial pharmacological target. No drugs currently have been approved for VP24. Based on these findings, driven by the effort to identify compounds able to inhibit VP24, we developed a new miniaturized dual drug screening assay to quantify the suppression of IFN cascade by VP24 and the effect of potential inhibitors. The assay is based on the transient cotransfection of HEK293T cells with a luciferase reporter under the control of the promoter of IFN stimulated genes (pISRE-luc), a plasmid expressing VP24 and RL-TK, as control of transfection efficiency. The stimulation with IFN-ř led to the activation of ISRE expression in cells transfected only with pISRE-luc. In contrast, cotransfection with VP24 led to the inhibition of ISRE transcription. Addition of compounds, such the same IFN-ř, led to the partial restoration of the pathway. We optimized the assay to achieve excellent signal and robust performance. Further, the normalization with a Renilla luciferase control allowed to minimize variability between experiments providing high reproducibility.

151. Favipiravir-resistant Chikungunya Virus is Severely Attenuated in Mosquitoes

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The chikungunya virus (CHIKV) is transmitted by *Aedes aegypti* and *albopictus* mosquitoes, mostly present in (sub) tropical regions. Several molecules with anti-CHIKV activity were recently reported and for some of these, resistant CHIKV variants could be selected in cell culture. However, no information is available about the replication and transmission abilities of these antiviral drug-resistant viruses in mosquitoes. We therefore infected *Aedes aegypti* mosquitoes via the oral route with wild-type CHIKV or a CHIKV variant that is resistant to favipiravir (T-705). The dissemination and transmission of the favipiravir^{res} CHIKV was markedly decreased as compared to WT. Intrathoracic virus injections showed that the midgut barrier of the mosquito is not solely responsible for the abrogated dissemination of favipiravir^{res} CHIKV. The infectious virus titers in mosquito bodies and heads were significantly lower for favipiravir^{res} CHIKV when compared to WT. The attenuated phenotype of the resistant variant was confirmed *in vitro* in the Aag2 and C6/36 mosquito cells at 28°C. Replication kinetics studies at 32°C for both Vero and mosquito cells confirmed that the attenuated fitness in mosquito cells is associated with the host and not with temperature. The transmission of a CHIKV variant carrying the key resistance mutation in the RdRp gene did not differ significantly from the transmission of WT, indicating that this mutation is not responsible for the attenuated phenotype. More research is ongoing to identify the molecular determinants that are involved in the attenuated phenotype of this variant, which may also provide important insights in the mosquito tropism of CHIKV.

152. New Antivirals for Bovine Viral Diarrhea Virus Selected by Virtual Screening against Viral RNA Polymerase

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Bovine viral diarrhea virus (BVDV) is a pestivirus whose infection in cattle is distributed worldwide. The use of antivirals could complement vaccination as a tool of control and reduce economic losses.

Viral RNA-dependent RNA polymerase (RdRp) is essential for viral genome replication and constitutes an attractive target for the discovery of antivirals. To obtain selective inhibitors of BVDV, around of 1400 compounds from HitFinder commercial databases and 230 compounds from the laboratory of Medicinal Chemistry (CIBION-CONICET) were screened in a high-throughput docking fashion. This led to the selection of several structurally different lead candidates that were either synthesized (S1-S5) or purchased (S6-S13). Their cytotoxicity in MDBK cells was assessed by MTS/PMS and the anti-BVDV (NADL) activity was evaluated by cytopathic effect reduction.

At least three compounds resulted active: S1, S7 and S9 with EC50 values of 9.68±0.49 μM (SI: 5.77); 0.98±0.01 μM (SI: 11.53); and 6.40±0.70 μM (SI: 12.90), respectively. For these compounds, molecular dynamics simulations characterized their possible binding determinants. A common pattern of interactions between active molecules and aminoacid residues in the binding site in RdRp was observed. Additionally, to improve the antiviral potency of quinazoline S1, whose synthesis is simple and easily scalable, eleven derivatives were synthesized and evaluated. As result, four of them showed improved antiviral activity, with EC50 ranging from 0.9 to 2.8 μM. In conclusion, these studies highlight the potential of virtual screening for the efficient discovery of new antivirals for BVDV. Further analysis will be done to validate their mechanism of action.

153. Direct Cell-To-Cell Spread can be Specifically Targeted by Small Molecule Inhibitors

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Many steps in viral replication are targeted by antivirals, including entry and egress. Although many viruses spread by direct cell-to-cell transmission, however, no antiviral specifically targets it.

The HIV RT inhibitory family of diarylpyrimidines are also active against other RNA or DNA viruses. Pursuing our interested in broad-spectrum antivirals, we tested a new family of diarylpyrimidines against DNA or RNA viruses, enveloped or not, with nuclear or cytoplasmic replication. Z214 affected only HCV, inhibiting foci formation (EC_{50}, 13 μM; SI, 4). The optimized Z390 inhibited HCV spread from infected to neighboring cells in the presence or absence of neutralizing antibodies (EC_{50}, 5 μM; SI, 45), but it did not inhibit single-step HCV replication, inactivate virions, render cells non-permissive to infection or replication, or inhibit plaque formation by dengue or Zika (flavi) virus. Z390 induced accumulation of HCV capsid and NS5A, but not NS3, to the surface of large lipid droplets (LD). Z390 induced an increase in mature, and decrease in nascent, LD in uninfected cells, as oleic acid (OA) does. OA also induced accumulation of NS5A and capsid to LD and inhibited HCV cell-to-cell spread. The small GTPase Arf1 regulates LD homeostasis. As Z390, Arf1 knockdown also inhibited HCV cell-to-cell spread, and GTP or GDP bound locked Arf1 mutants inhibited the accumulation of capsid and NS5A to LD.

New diarylpyrimidines inhibit HCV cell-to cell spread by modulating the required LD homeostasis, effect mimicked by locking Arf1 in GTP or GDP bound states. Direct cell-to-cell viral spread can be an antiviral target.

155. Targeting Histone Deacetylation: Effects on Host Inflammation and Lung Pathology in Influenza A/H3N2-Infected Mice

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The conventional influenza treatment often fails against changeable virus nature facing up rapid selection and spread of resistance, adverse reactions and drugs' cost, as well. Acting on the host defense mechanisms at epigenetic level may alter delicately the cell-dependent mechanisms of viral propagation.

Herein we have exploited histone deacetylases (HDACs) as epigenetic targets for modulation of lung inflammation during influenza infection. We studied the effect of selective HDAC inhibitor Trichostatin A (TSA) on the severity of influenza. TSA was administered intraperitoneally at a dose of 10 mg/kg/day to ICR mice in a 5 day-lasting course. We evaluated survival, lung viral titers, and pulmonary changes including cell infiltration. A group treated with 10 mg/kg/day referent oseltamivir phosphate served to compare the antiviral responses *in vivo*.

We found that TSA failed to exert protective effect in experimental influenza A H3N2-infected mice. The survival of TSA-treated and infected animals was lower than in the placebo control. Indeed, TSA administration increased lung index indicative for severe exudate formation 2 times more as compared to the reference antiviral. Lung viral titer in TSA-treated mice with flu exceeded that in the untreated infected mice by 0.67 Lg. Correspondingly we observed alterations in the histological score indicative for lung inflammation and cell infiltration. In a control group the dose of 10 mg/kg oseltamivir phosphate showed 80% mice survival, decreased lung pathology and lung virus titer.

Our data showed that the inhibition of HDACs may have detrimental effects during influenza-virus infection by altering lung pathology and inflammation.

156. Antiviral Efficacy of Tilorone Dihydrochloride against Bunya- and Paramyxoviruses

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Tilorone dihydrochloride (tilorone) is a small molecule that is an approved antiviral therapeutic outside the United States. It has been reported to inhibit many unrelated viruses, such as influenza A and B viruses, West Nile virus, herpes simplex virus, and Venezuelan equine encephalitis virus. Most recently, its antiviral activity has also been demonstrated for Ebola virus in a mouse model. Here, we investigated the antiviral activity of tilorone against bunyaviruses (Rift Valley fever virus, RVFV) and paramyxoviruses (measles virus, Nipah virus) to further characterize its broad-spectrum antiviral efficacy. Cytotoxicity, plaque reduction (PR) and virus yield reduction (VYR) assays were performed in Vero and A549 cells. The purine analogue favipiravir was included as positive control. In PR assays in Vero cells, tilorone inhibited RVFV with an EC_{50} of 0.05μM, and in a VYR assay of 0.8μM. In interferon-competent A549 cells, the EC_{50} was 1.6μM in a VYR assay. In contrast, favipiravir was inhibitory at 9μM. In time-of-addition experiments using a recombinant RVFV expressing GFP, near complete suppression of viral replication was observed when tilorone was added to cells up to six hours post infection. Pre-treatment of cells with tilorone prior to infection resulted in suppression of viral replication. Tilorone was also evaluated for its antiviral efficacy against paramyxoviruses, resulting in EC_{50}'s of 1.5μM for measles virus, and 10μM for Nipah virus. Our data demonstrate that tilorone has antiviral activity against members of the order bunyavirales and paramyxovirus family, further strengthening the broad-spectrum antiviral potential of tilorone.

157. Crystallography-Based Drug-Like Fragment Screening with Polyomavirus VP1

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Closely related BK and JC polyomaviruses can cause severe clinical complications in solid organ and hematopoietic transplant recipients as well as in those patients on immunomodulatory agents. While BKPyV replication has been associated with the risk of acute allograft rejection, current therapeutic landscape of polyomavirus infection is dismal with no approved therapies. Polyomavirus capsid protein VP1 plays a key role in viral assembly and pathogenesis. 72 pentamers of VP1 ultimately assemble to form a T=7 icosahedral virion which protects the viral genome. Capsid assembly inhibitors have been proposed in the past for several viruses including HIV and hepatitis B and C viruses. Here we report progress into a structure-guided design of inhibitors targeting the BK/JC polyomavirus capsid. After optimizing VP1 purification and crystallization, we conducted an extensive X-ray crystallography-based screen of over 1000 drug-like fragments, made possible through the XChem facility at Diamond Light Source, UK. We could identify several novel druggable pockets accommodating multiple fragments. One induced pocket is particularly attractive as it overlaps with the site previously shown to be important for capsid assembly. This pocket exhibits sequence and structural conservation across four BK strains and JC virus. Structure-guided fragment growth and optimization are ongoing.

158. Ouabain Inhibits Influenza A Viruses Replication

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Influenza A virus has been threatening the public health by emerging of avian, pandemic and seasonal influenza viruses. Antiviral agents are essential for the treatment of influenza infection. However, current anti-influenza drugs have some limitations: high frequency of resistance to M2 inhibitors, appearance of neuraminidase inhibitors (NAI)-resistant virus and insufficient efficacy to cases of severe illness. Thus, it is necessary to develop alternative drugs to compensate the drawbacks of current antivirals. In order to overcome these limitations, we assessed antiviral activity of ouabain (OUA), Na+/K+ ATPase inhibitor, against influenza A viruses infection. OUA treatment drastically inhibited that viral mRNA levels (NP, M1 and HA) at 6 hour post-infection (hpi). Accordingly, viral NP, M1, and NS1 protein expression in the infected cells were significantly inhibited until 8 hpi. In mouse model, survival of OUA treated group (50%) was comparable to that of zanamivir (60%) after highly pathogenic avian H5N1 virus infection. These results indicated that OUA could be a novel inhibitor of influenza viral replication. However, further study is necessary to elucidate the antiviral mechanism. This research was supported by a fund by Research of Korea Centers for Disease Control and Prevention (Grant number: 2016-NI43001-00).

159. Novel Diphosphate Analogues of PMEA: Synthesis and Study of their Ability to Inhibit HIV-RT

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A series of diphosphate analogues of the antiviral agent 9-[(2-phosphonomethoxy)ethyl]adenine (PMEA, Adefovir) bearing non hydrolysable bonds between the beta and gamma-phosphorus atoms was designed as potential substrates and/or inhibitors of viral polymerases. Indeed, previous studies based on AZT and 2',3'-dideoxynucleosides, as models, have shown that replacement of beta,gamma-pyrophosphate by beta,gamma-phosphonate moieties within the triphosphate chain may retain the terminating substrate properties of these analogue towards HIV-RT. Synthesis involved the coupling of a morpholidate derivative of PMEA with appropriate pyrophosphoryl analogues. Then, their substrate properties were studied towards HIV-1 reverse transcriptase in comparison to the parent nucleotide analogue (PMEApp). Only one of them was recognized as terminating substrate but it proved to be less effective than PMEApp.

160. Potent and Broad-Spectrum Small Molecules that Disrupt the PA-PB1 Subunits Interaction of Influenza Virus RNA Polymerase and Possess a High Genetic Barrier to Drug-Resistance

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Influenza virus represents a serious threat for public health as it causes seasonal outbreaks worldwide that can lead to severe illness or death. Great concern has recently arisen over the continuous zoonotic infections of humans with avian influenza and their pandemic potential. The limited available therapeutic options along with the rapid emergence of drug-resistance make essential the development of alternative and more effective anti-influenza drugs. In the last years, we developed several compounds able to disrupt the interactions between the PA-PB1 subunits of influenza virus RNA polymerase and to inhibit virus replication and have proposed a pharmacophoric model. Taking advantage of the information emerged from the pharmacophore, we developed a series of potent PA-PB1 inhibitors, endowed with sub-micromolar antiviral activity. Selected compounds inhibited in a dose-dependent manner the replication of a panel of influenza A and B strains with similar potency in plaque reductions assays. Potent antiviral activity was then confirmed by virus yield reduction assays. These compounds also induced a dose-dependent reduction of PA-PB1 binding in ELISA interaction assays and caused a strong impairment of the catalytic activity of influenza A virus polymerase in minireplicon assays. Moreover, *in vitro* resistance studies indicated that these inhibitors have a higher genetic barrier to drug-resistance than oseltamivir. Indeed, unlike oseltamivir, the most promising compounds did not induce the selection of resistant viral strains after serial passages of an influenza virus under increasing drug concentrations. Taken together, these results support our PA-PB1 inhibitors as promising antiviral candidates for treating influenza virus infections.

161. Combinations of Terminase Inhibitors Elicit a Synergistic Enhancement of Effect against Human Cytomegalovirus *In Vitro*

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Human cytomegalovirus (CMV) infections can be detrimental to patients with compromised or immature immune systems. Complications that may arise from a CMV infection include gastroenteritis, interstitial pneumonia, retinitis, and developmental abnormalities. The current first-line pharmacotherapy option for treating CMV is ganciclovir, a nucleoside analog prodrug that targets the viral DNA polymerase (pUL54) resulting in viral DNA synthesis inhibition. However, this drug suffers from two major drawbacks: dose-limiting toxicity (specifically neutropenia) and drug resistance. Therefore the development of new compounds with greater therapeutic indices and/or novel mechanisms of action are warranted. Letermovir, BDCRB, UMJD1853, and UMJD1896 are drugs that target the CMV terminase complex. Previous studies have determined that letermovir targets pUL56, the terminase subunit that binds DNA, interacts with portal protein (pUL104), and provides energy for DNA translocation and cleavage. BDCRB,

UMJD1853, and UMJD1896 target pUL89, the terminase subunit that is responsible for cleaving CMV concatemer DNA into individual genomic units. Since these drugs have distinct binding sites, we hypothesize that the combination of Letermovir and BDCRB/UMJD1853/UMJD1896 will demonstrate an additive or synergistic enhancement of effect against CMV. Our results demonstrate that the combination of Letermovir and BDCRB, UMJD1853, or UMJD1896 have drug combination index numbers of 156, 160, and 141 with 95% confidence intervals of 224-88, 251-69, and 219-63, respectively. Since these values are statistically greater than zero, we conclude that the combination of two terminase inhibitors (Letermovir, and BDCRB, UMJD1853, or UMJD1896) results in a synergistic enhancement of antiviral effect against CMV *in vitro*.

162. The Inhibitory Effect of Mycophenolic Acid Derivatives on MERS Coronavirus Papain-Like Protease

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Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by the MERS coronavirus(MERS-CoV). The first identified case emerged in 2012 in Saudi Arabia and then spread to other countries in recent years. The symptoms of MERS include fever, cough, diarrhea, shortness of breath and even worse in some cases. About 3-4 out of every 10 patients reported with MERS have died, which has resulted in an urgent need to identify antiviral drugs that can target and inhibit MERS-CoV. Previously, our lab found that mycophenolic acid (MPA) and 6-thioguanine (6TG) can synergistically inhibit MERS-CoV papain-like protease(PL^{pro}), which is one of the two proteases from MERS-CoV responsible for virus maturation. Recently, we have synthesized several MPA derivatives which were conjugated with 6TG and its analogs, and analyzed their inhibitory effect on MERS-CoV PL^{pro}. Further study on the inhibition mechanism is ongoing and we believe that the information will benefit the next round's drug optimization.

163. Env-Driven Replicative Fitness Seems to be Associated with a Reduced Response to Cart in Patients Infected with HIV-1 Subtype F Strains

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BACKGROUND: We previously reported an impaired response to antiretroviral treatment (ART) in patients infected with subtype F compared to individuals infected with subtype B HIV-1 strains. Here, we characterized a series of subtype F and B HIV-1 strains from NW Spain patients to better understand the mechanism(s) associated with these findings.

METHODS: Plasma samples from patients infected with subtype F (10) or B (10) viruses were obtained. Two sets of recombinant viruses (3'Gag/PR/RT/INT and env) were constructed and used in drug susceptibility, neutralization assays, VGK and competition experiments. Deep sequencing of full-length HIV-1 genomes was used to determine drug resistance, tropism, and intrapatient HIV-1 diversity.

RESULTS: Plasma viremia at baseline (5.65 vs. 4.91 log c/ml, p=0.013) and response to cART (<50 log c/ml; 49 vs. 20 weeks, p=0.026) was higher in the subtype F group. All 20 HIV-1 strains were susceptible to all ARV drugs. No differences in tropism was observed and all viruses were equally neutralized by two bNABs. Interestingly, subtype F env-recombinant viruses showed slightly higher fitness compared to subtype B viruses (mean 0.036 vs. 0.015, p=0.081). Intrapatient HIV-1 diversity was also slightly higher in individuals infected with subtype F viruses (1.08 vs. 0.89, p=0.37). Full HIV-1 genome analysis identified 39 polymorphisms present in subtype F but absent in all subtype B viruses

CONCLUSIONS: The significant delay in initial response to cART in patients infected with subtype F HIV-1 strains seems to be associated with higher viral replication capacity, most likely driven by the *env* gene.

165. Disrupting Viral Transcriptional Regulatory Circuitry Constitutes an Escape Resistant Therapeutic Strategy

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Cytomegalovirus (CMV), one of the eight known human Herpesviruses, infects most of the world population and is a leading cause of birth defects in the United States and causes life-threatening diseases in immune-compromised individuals (eg. transplant recipients). Current antiviral therapies for CMV face several obstacles including dose-limiting toxicity and the presence of drug-resistant strains. Hence, there is an unmet medical need for new CMV antivirals. We

designed a new class of oligonucleotide therapy, which specifically targets and disrupts viral transcriptional circuitry resulting in >2 log decreases in virus replication without apparent host-cell toxicity. This strategy of targeting viral transcriptional circuitry appears to be robust to the evolution of viral resistance and broad spectrum, extending to other herpesviruses.

166. Mapping the Mutations in HCV Protease Conferring Resistance to Grazoprevir or Danoprevir

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Hepatitis C virus (HCV) infects more than 185 million people worldwide, which is a leading cause of chronic liver disease, liver cirrhosis and hepatocellular carcinomas. Remarkable achievements have been made in the treatment of hepatitis C with direct-acting antivirals (DAAs), while mutations in the protease NS3/4A or polymerase NS4B have been reported to be associated with drug resistance, which is a major obstacle to therapeutic success. It is challenging to identify any mutation in NS3/4A or NS5B of HCV conferring drug resistance. However, knowledge about drug resistance conferring mutations is essential to combat drug resistance against HCV DAAs as well as to develop new highly effective antivirals in the future. We found that the HCV protease inhibitors Grazoprevir and Danoprevir significantly inhibited the cleavage of MAVS by NS3/4A *in vitro*, but not Telaprevir, Boceprevir or Ciluprevir based on a real-time live-cell reporter. We developed a novel CRISPR-based approach to map all the potential mutations in NS3/4A conferring resistance to Grazoprevir or Danoprevir.

167. A Drug Repurposing Approach Identifies Different Approved Compounds that Specifically Inhibit Human Cytomegalovirus (HCMV) Replication with Mechanisms Different from that of the Current Anti-HCMV Drugs

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Currently, only few drugs are licensed to treat human cytomegalovirus (HCMV) infections, a major threat for immunocompromised patients and pregnant women. To identify alternative targets of therapeutic intervention, we applied a strategy of drug repurposing using a cell-based screening that allows the identification of inhibitors of the transactivating activity of the Immediate Early-2 (IE-2) protein, an essential virus-encoded transcription factor. We identified a series of compounds, including some already approved drugs in clinical therapy for the treatment of other diseases, that could represent new therapeutic agents to treat HCMV infections in alternative to the currently available DNA polymerase and terminase inhibitors. Here, we describe the potential to repurpose nitazoxanide (NTZ), an antiparasitic drug, and manidipine dihydrochloride (MND), an anti-hypertensive calcium antagonist, as new anti-HCMV agents. Both drugs inhibited the replication of different HCMV strains, including viruses resistant to approved anti-HCMV drugs, in the low micromolar range. Further experiments testing the effects of NTZ and MND on viral DNA synthesis and on viral proteins expression revealed that they halt the progression of the virus cycle prior to viral DNA replication and E genes expression, but after IE proteins expression. Accordingly, we observed that the antiviral activity of both molecules involves a specific interference with IE-2 protein. Given that the inhibitory concentrations against HCMV fall in the range of clinically relevant concentrations of these drugs in humans and their mechanism of action differs from that of the other available antivirals, these compounds are attractive candidates for repurposing in alternative anti-HCMV therapeutic protocols.

168. CD32 Expression is Associated to T Cell Activation and Upregulated by HIV

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The overexpression of the gene encoding for the transmembrane protein FCGR2A (CD32a+) has been proposed as a potential marker of HIV+ latently infected cells. We aim to identify the role of CD32 as molecular signature of resting, latently infected cells to facilitate the development of new therapeutic approaches. CD32 and activation markers HLA-DR and CD69 were measured in PBMC and CD4+ T lymphocytes from HIV+ individuals or healthy donors stimulated with or without IL-2 (16 U/ml), PHA (4 µg/ml), řCD3/CD28 or IL-7 (10 ng/ml) and/or infected with an HIV-1 NL4-3-GFP virus carrying Vpx. Contribution of CD32+ cells to the viral reservoir was determined in sorted CD4+ T cells from healthy donors infected *in vitro* or HIV+ patients by qPCR of integrated HIV-1 DNA.

Activation of CD4+ T cells irrespective of the stimuli, upregulated CD32, HLA-DR and CD69. Productive infection of CD4+ T cells increased CD32 expression in CD4 T cells. CD32 expression in CD4+ T cells from HIV+ individuals under antiretroviral treatment indicated that a mean of 85% of cells were CD32+/HLA-DR+. Proviral DNA copies/cell in resting was higher in CD4+/CD32- T cells infected with HIV-1 NL4-3*GFP-Vpx. There were no statistically significant differences in the mean viral DNA copies/cell in CD32+ or CD32- CD4+ T cells from HIV+ individuals under therapy. In conclusion, CD32 expression is a marker of CD4+ T cell activation in healthy donors and HIV+ patients. The viral reservoir lay outside the CD32+ component and therefore HIV-1 latency may not be preferentially associated to CD32+ cells.

169. Synthesis of Non-Hydrolysable "Super Substrate" of Phosphatidylinositol 4-Kinases, Important Players in Host-Virus Interaction

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Phosphatidylinositol 4-kinases (PI4Ks) are enzymes responsible for the transfer of phosphate group from ATP to phosphatidylinositol, a common constituent of the plasma membranes in cells. The product of this reaction, phosphatidylinositol 4-phosphate (PI4P), plays numerous roles in cell signalling and is essentially implicated in process of membrane budding. In human cells, there are four different PI4Ks, divided into two classes – class II, which includes PI4K2A and PI4K2B, and class III containing PI4KA and PI4KB. Both members of class III are well-known host factors implicated in replication of various RNA viruses. PI4KA and PI4KB play an essential role in the life cycle of hepatitis C virus (HCV). These PI4Ks are necessary for the reorganization of cellular membranes indispensable from the formation of replication organelles. In contrast, members of Picornaviridae family exploit only PI4KB for these purposes. Although crystal structures of PI4K2A, PI4K2B and also PI4KB with nucleoside and non-nucleoside inhibitors were reported, so far, there is no crystal structure unveiling the position of the phosphatidylinositol within the active site. We report the design and synthesis of novel "super substrate" complex of ADP and PI4P containing non-hydrolysable bisphosphonate motive. Since such "super substrate" can, in principle, mimic also the transition state of the phosphorylation reaction, this compound should inhibit the function of PI4Ks as well as replication of viruses, which utilize these host enzymes for the shaping of replication organelles.

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170. Anti-Varicella Zoster Virus Activity of Amenamevir for Treatment of Herpes Zoster

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Amenamevir is a novel helicase-primase inhibitor of herpes simplex virus and varicella-zoster virus. Amenamevir has been approved for the treatment of herpes zoster in September 2017 and so far about 100,000 patients has been treated in Japan. Helicase-primase (HP) inhibitors inhibit progression of the replication fork, an initial step in DNA synthesis to separate the double strand into two single strands. We have characterized the effects of the viral replication cycle on the antiviral action of amenamevir in comparison with acyclovir, sorivudine, and forcarnet. The concentrations of amenamevir and foscarnet effective for 50% plaque reduction (EC50) were similar at 0 h and 18 h after cell-free virus infection, but those of acyclovir and sorivudine were 14 and five times, respectively, larger at 18 h than at 0 h after infection. The direct inhibitions of HP by amenamevir and DNA polymerase by foscarnet contrasted with the indirect inhibition of DNA synthesis by the triphosphate forms of acyclovir and sorivudine, which are phosphorylated by viral thymidine kinase; the latter inhibition was attenuated by the supply of guanosine- and thymidine-triphosphates, respectively, when they were supplied through viral ribonucleotide reductase and thymidylate synthase in the replication phase of infection. We observed superior character of amenamevir in suppressing viral growth in the viral DNA synthesis stage over the current anti-VZV agents.

171. Indolylarylsulfones with Potent Anti-HIV-1 Activity

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Acquired immune deficiency syndrome (AIDS) pandemic remain among the leading causes of death worldwide. HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTIs) are key drugs of highly active antiretroviral therapy (HAART) in the clinical management of AIDS/HIV-1 infection.

We designed and synthesized a series of chiral indolyarylsulfones (IASs) as new HIV-1 NNRTIs (1). The new IASs showed potent inhibition of the HIV-1 WT NL4-3 strain and of the mutant K103N, Y181C, Y188L, and K103N–Y181C HIV-1 strains. Six racemic mixtures, 8, 23 and 25, 31 were separated at semipreparative level into their pure enantiomers. The (R)-8 enantiomer bearing the chiral (ř-methylbenzyl) was superior to the (S)-counterpart. IAS derivatives bearing the (S) alanine unit, (S)-23, (S,R)-25, were remarkably more potent than the corresponding (R)-enantiomers. Compound 23 protected hippocampal neuronal cells from the excitotoxic insult, while efavirenz (EFV) did not contrast the neurotoxic effect of glutamate. The present results highlight the chiral IASs as new NNRTIs with improved resistance profile against the mutant HIV-1 strains and reduced neurotoxic effects.

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172. Metabolic Activation of MBX-2168 Involves Enzymatic Removal of a Butyl-Ether Moiety by Adenosine Deaminase-Like Protein 1

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Ganciclovir is the primary pharmacotherapy option for the treatment of systemic cytomegalovirus (CMV) infections. The mechanism of action for GCV is monophosphorylation by the CMV-encoded pUL97 kinase followed by endogenous cellular phosphorylation to a triphosphate, the active compound that inhibits viral genome replication. However, the emergence of drug resistant virus and a high incidence of adverse effects limit the utility of this drug. MBX-2168 demonstrates increased potency against CMV without any observable increase in cytotoxicity. The mechanism of action for MBX-2168 is identical to ganciclovir with two additional and distinct steps. Previous studies have demonstrated that MBX-2168 is partially phosphorylated to a monophosphate by the endogenous cellular kinase TAOK3. This first distinct metabolic step allows MBX-2168 to overcome resistance associated with mutations in the virus-encoded pUL97 kinase. Our current studies demonstrate that the addition of pentostatin, an adenosine deaminase-like protein 1 (ADAL1) inhibitor, antagonizes the anti-viral activity of MBX-2168. We therefore hypothesize that the second distinct metabolic step in the activation of MBX-2168 involves the enzymatic removal of a butyl-ether moiety at the 6 position of the guanine ring by ADAL1. Incubation of MBX-2168 with cellular extracts following subjection to an ADAL1-baculovirus construct demonstrates conversion of MBX-2168-MP to synguanol-MP, a previous generation compound, to a greater extent when compared to extracts subjected to a construct void of the ADAL1 gene. In addition, studies using virus-infected cells demonstrate that these distinct metabolic steps do not alter the kinetics of activation. Studies to further characterize this metabolic step are ongoing.

174. Biological Evaluation of Novel Small-Molecule Antiviral Agents versus Tick Borne Encephalitis Virus

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Tick Borne Encephalitis Virus (TBEV) is a flavivirus causing a flu-like illness and meningoencephalitis in the human host. TBEV is transmitted by Ixodes ticks and endemic in temperate climate zones suitable for the vector, mainly in Europe and Asia, but not in the Americas. A number of approved inactivated TBEV vaccines are on the market, but so far, there are no TBEV specific therapeutics.

A number of compounds previously found active versus flaviviruses and non-toxic in human HUH7 hepatoma cells, were tested versus TBEV in human cell lines where TBEV causes cytopathogenic effects (CPE). Several compounds with IC50 in the uM range and minimal toxicity were identified.

Further biological evaluation of these compounds in different cell lines, including central nervous system cells, and different assay methods is ongoing and will be reported.

This work provides the foundation for further investigation of promising novel structures as antiviral agents against TBE virus.

175. Antiviral Synergy between Zika Virus Protease and Polymerase Inhibitors

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Zika virus (ZIKV) infection may be associated with congenital malformations in infected fetuses and severe complications in infected adults. There are limited treatment options that are readily available. We previously reported the *in vitro* and *in vivo* antiviral activity of novobiocin against the closely related Zika virus through inhibition of the Zika virus NS2B-NS3 protease. In this study, we investigated the antiviral activity of combinational novobiocin (protease inhibitor) and ribavirin (polymerase inhibitor) against ZIKV. The *in vitro* anti-ZIKV activities of novobiocin and ribavirin were individually assessed in multiple cell lines using cytopathic effect inhibition, viral load reduction, and plaque reduction assays, followed by checkerboard assay for combinational effect. While both drugs significantly inhibited ZIKV *in vitro* when given alone, their combinational effect was synergistic (Loewe additivity index <1). Molecular docking predicted that novobiocin and ribavirin bound to the ZIKV protease and polymerase, respectively, with high stability. Our study showed that combinational treatment targeting multiple enzymes of ZIKV achieved better *in vitro* anti-ZIKV activity. Further studies should be conducted to explore other clinically available combinations of ZIKV enzyme inhibitors to facilitate the development of effective treatments against this emerging infection.

176. Identification of Combinations of Approved Drugs with Synergistic Activity against Ebola Virus in Cell Cultures

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A need to develop therapeutics to treat Ebola virus disease patients in remote and resource-challenged settings remains in the wake of the 2013–2016 epidemic in Western Africa. Towards this goal, we screened drugs under consideration as treatment options and other drugs of interest, most being small molecules approved by the Food and Drug Administration. Drugs demonstrating *in vitro* antiviral activity were advanced for evaluation in combination due to advantages often provided by drug cocktails. Drugs were screened for blockade of Ebola virus infection in cultured cells. Twelve drugs were tested in all (seventy-eight pairwise) combinations, and three were tested in a subset of combinations. Multiple synergistic drug pairs emerged, with the majority comprised of two entry inhibitors. For the pairs of entry inhibitors studied, synergy was demonstrated at the level of virus entry into host cells. Highly synergistic pairs included aripiprazole/piperacetazine, sertraline/toremifene, sertraline/bepridil and amodiaquine/clomiphene. Our study shows the feasibility of identifying pairs of approved drugs that synergistically block Ebola virus infection in cell cultures. We discuss our findings in terms of the theoretic ability of these or alternate combinations to reach therapeutic levels. Future directions will be aimed at assessing selected combinations in small animal models of Ebola virus disease.

177. Metal Chelating Agents against Viruses

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Influenza viruses cause considerable morbidity and mortality, whether in the context of annual epidemics, sporadic pandemics, or outbreaks of avian influenza virus. For hepatitis C virus, an estimated 170 million people are chronically infected worldwide and are at high risk of developing progressive liver injury or hepatocellular carcinoma. On the other hand, hepatitis B virus chronically infects >250 million people and kills nearly a million annually. Moreover, the flaviviruses Dengue, Yellow fever and West Nile are high priority targets for drug discovery, as they are reemerging global pathogens with no clinically approved specific therapy. Since emerging viral resistance remains high, the cost threaten the efficacy of currently approved antiviral drugs and the attention of pharmaceutical industry concerning neglected and relatively unprofitable virus disease is little, new antiviral drugs are urgently needed.

Approximately one-third of proteins are metalloproteins, some of them are responsible for a wide variety of essential viral functions. Given the impact of the aforementioned infectious diseases on human health, metalloenzyme inhibition offers an appealing approach to disease treatment. Hydroxamates act as bidentate ligands and are easily coordinated with almost any enzyme that contains M^{2+} ion. Thus, the rational design-'structural tuning' of novel hydroxamates, with new and dual mode of action, such as single derivative that may act on two different viruses and target enzymes, is a very intriguing approach and attracts pharmaceutical industry's interest, as it would limit the number of administered drugs, their chronic toxicity and the possibility of developing drug resistant viruses.

178. Mapping Influenza Hemagglutinin Evolutionary Conservation to its Structural Plasticity: When sequence and structural dynamics can elucidate conserved epitope accessibility

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Influenza hemagglutinin fitness selection is likely constrained between maintaining its structural similarity for successful attachment and fusion while also continuously evading host detection, and yet the structurally-imposed limitations on hemagglutinin sequence evolution are not fully characterized. We use a protein design method, Rosetta RECON, to determine the hemagglutinin evolutionary sequence profile to illustrate regions of high and low sequence variation. We show that this design approach mimics observed subtype residue-specific sequence variation, with key exceptions that favor post-fusion stabililty, suggesting that this method is a suitable approach for not only identifying sequence profiles suitable for the large conformational rearrangement hemagglutinin assumes between attachment and fusion, but also mutations that possibly stabilize specific conformations. We then performed accelerated molecular dynamics simulations of the glycosylated hemagglutinin trimer using multiple pH environments to illustrate the concerted rearrangement of the head and stem regions. By mapping sequence conservation profiles onto the simulated trajectories, we identify changes in surface accessibility of highly conserved regions, which can be used to identify possible conserved epitopes that are accessible during intermediate stages of fusion.

179. Nanobodies Reveal Functional Epitopes and Potential Mechanisms of Norovirus Neutralization

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Norovirus is the leading cause of gastroenteritis worldwide. Little is known on how norovirus infects the host cells, except that histo-blood group antigens (HBGAs) are important binding factors for infection and cell entry. Antibodies that bind at the HBGA pocket and block attachment to HBGAs are believed to neutralize the virus. However, additional neutralization epitopes elsewhere on the capsid likely exist and impeding the intrinsic structural dynamics of the capsid could be equally important. In the current study, we investigated a panel of Nanobodies in order to probe functional epitopes that could trigger capsid rearrangement and/ or interfere with HBGA binding interactions. The precise binding sites of eight Nanobodies were identified using X-ray crystallography. We showed that these Nanobodies bound on the top, side, and bottom of the norovirus protruding domain. We discovered that distinct Nanobody epitopes were associated with varied changes in particle structural integrity and assembly. Interestingly, certain Nanobody-induced capsid morphological changes lead to the capsid protein degradation and viral RNA

exposure. Moreover, Nanobodies employed multiple inhibition mechanisms to prevent norovirus attachment to HBGAs, which included steric obstruction (Nano-14), allosteric interference (Nano-32, Nb94), and violation of normal capsid morphology (Nano-26 and Nano-85). Finally, we showed that two Nanobodies (Nano-26 and Nano-85) not only compromised capsid integrity and inhibited VLPs attachment to HBGAs, but also recognized a broad panel of norovirus genotypes with high affinities. Consequently, Nano-26 and Nano-85 have a great potential to function as novel antiviral agents against human noroviruses.

180. *N4-p*-nitrophenylthiosemicarbazone of 5,6-dimethoxy-1-indanone as a New Non Nucleoside Inhibitor of BVDV RNA Polymerase

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Bovine viral diarrhea virus (BVDV) causes important economic losses to the livestock industry and has no specific treatment. The thiosemicarbazone of the 5,6-dimethoxy-1-indanone (5,6-TSC, EC50=3.8μM) was previously reported by our group as an anti-BVDV agent. After structural optimization, the *N4-p*-nitrophenylthiosemicarbazone of the 5,6-dimethoxy-1-indanone (15) presented the highest *in vitro* antiviral activity (EC50=0.7μM; S.I.>21.4).

In order to characterize its mechanism of action, (a) virucidal, (b) cell-pretreatment, and (c) time-of-(drug)-addition assays were performed, using BVDV-1 NADL strain and MDBK cells. For (c), extracellular viral production (PFU/mL) and intracellular viral RNA (q-RT-PCR) were determined. In addition, BVDV resistant viruses were selected by successive biological cloning steps in the presence of 15. The NS5B region of the viral genome, which encodes for the RNA-dependent-RNA-polymerase (RdRp), was amplified by RT-PCR and sequenced.

Neither virucidal activity nor cell-pretreatment effect were observed. In the time-of-(drug)-addition assay, maximum inhibition was observed when **15** was added within the first 6 h post-adsorption, time point which coincides with the onset of the viral RNA synthesis. Moreover, five BVDV resistant variants (15^r BVDV; EC_{50}>15 μM) were selected. A N264D change in the RdRp was found in all of them, and has been also associated to resistance for 5,6-TSC and other anti-BVDV agents. In addition to molecular docking results, these findings suggest that 15 acts as a non nucleoside inhibitor of BVDV RdRp.

To further characterize 15 biological activity, evaluation of other BVDV strains and RNA viruses is currently in progress. Results will be presented during the meeting.

181. Hepatitis B Virus Core Protein Dephosphorylation is Required for Pregenomic RNA Encapsidation

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Hepatitis B virus (HBV) core protein consists of N-terminal assembly domain and C-terminal domain (CTD) with seven conserved serines or threonine that are dynamically phosphorylated/dephosphorylated during the viral replication cycle. Sulfamoylbenzaminde derivatives are small molecular core protein allosteric modulators (CpAMs) that bind to the HAP pocket between the core protein dimer-dimer interfaces. CpAM binding alters the kinetics and pathway of capsid assembly and results in the formation of morphologically "normal" capsids devoid of viral pregenomic (pg) RNA and DNA polymerase. In order to investigate the mechanism underlying CpAM inhibition of pgRNA encapsidation, we developed an immunobloting assay that can resolve core protein based on its phosphorylation status and demonstrated, for the first time, that core protein is hyperphosphorylated in free dimers and empty capsids from both mock-treated and CpAM-treated cells, but is hypophosphorylated in pgRNA- and DNA-containing nucleocapsids. Interestingly, inhibition of cellular phosphatases with okadaic acid significantly reduced the amounts of dephosphorylated core protein and encapsidated pgRNA. Moreover, core proteins with point mutations at the wall of HAP pocket, V124A or V124W, assembled empty capsids and nucleocapsids with altered phosphorylation status.

The results thus suggest that core protein dephosphorylation is required for the assembly of pgRNA and interference of the interaction between core protein subunits at dimer-dimer interfaces during nucleocapsid assembly not only alters capsid structure, but also core protein dephosphorylation. Hence, inhibition of pgRNA encapsidation by CpAMs might be due to disruption of core protein dephosphorylation during nucleocapsid assembly.

182. Development & Characterization of Poly ξ-Caprolactone Nanoparticles for Vaccine Delivery

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Worldwide more than 300 million peoples are infected with the Hepatitis-B virus(HBV) and more than 1 million people die each year of liver failure hepatocellular carcinoma(HCC). The aim is to prepare controlled release hepatitis-B antigen containing poly ξ-caprolactone (PCL) based nanoparticulate system that provide a long-circulating antigen reservoir from which can be released into the vascular compartment in a controlled manner.Nanoparticulate system have an adjuvant properties, they are used for the antigen targeting to dendritic cell and activate dendritic cells to induce HBV antigen specific T-cell response.

In the present study, PCL nanoparticles were prepared bydouble emulsion(w/o/w) solvent evaporation method. Hepatitis-B surface antigen(HBsAg) was selected as model antigen to optimize formulation & process variables. The formulations were optimized on the basis of particle size & entrapment efficiency. The external morphology of the optimized formulation was studied by SEM & TEM. Stability studies were performed at accelerated temperatures. The in-vivo study included fluorescence microscopy and estimation of serum for antibody titres.

The results show that mice immunized with intramuscular injection of nanoparticles loaded HBsAg produced higher immune responses as compared to the marketed preparation.

In conclusion, we have demonstrated that hydrophobic nature of PCL provides danger signals for dendritic cells and activated, so PCL based nanoparticles a valuable means of delivering a soluble antigen to dendritic cell and provides enormous potential in developing new vaccines against hepatitis B.

183. Studies on the Mechanism of Bacteriophage Mediated Inhibition of Human Adenovirus Type 5 Infection *In Vitro*

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In our previous experiments we have observed a significant reduction of human type 5 adenovirus (HAdV-5) titer in the presence of two bacterial viruses (*E. coli* T4 and staphylococcal A5W/80 phage). The aim of the study was to assess the influence of preparations containing T4 or A5/80 phage on adenoviral DNA level in *in vitro* cultures.

Adenovirus was propagated in A549 cell line and its two different titers were used for infection. Cells infected with HAdV-5 were incubated with purified phage preparations at an effective dose calculated on the basis of the previous experiments. Time-dependent experiments (adsorption and replication assays) were performed to measure a number of HAdV-5 DNA copies in the infected cells (8, 16, 32, 40 and 48 hours after infection) with the use of quantitative real time PCR. The highest reduction of adenoviral DNA level was observed in the cells treated with T4 phage 48 hours after infection (by 1.08-1.17 log of HAdV-5 DNA copy number, $P<0.01$ when higher viral infectious titer was applied). There was no significant impact of A5W/80 phage on adenoviral DNA level.

Results of this study suggest that inhibitory effect of T4 phage on HAdV-5 infection may be partially explained by its influence on synthesis of adenoviral DNA within A549 cells, while the antiviral effect of staphylococcal phage requires further investigation.

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184. Influenza A/H3N2 Virus Infection in Immunocompromised Ferrets and Emergence of Antiviral Resistance

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Influenza viruses can cause life-threatening infections in high-risk patients, including young children, the elderly and patients with compromised immunity due to disease complications or immunosuppressive treatment. The impaired immunity of these patients promotes prolonged virus infection and combined with antiviral treatment facilitates the emergence of viruses with resistance mutations. The diverse nature of the immune status of immunocompromised patients makes them a challenging group to asses optimal anti-influenza virus therapies. Immunocompromised ferrets represent a suitable animal model to study both the course of influenza virus infection and assess the efficacy of antiviral treatment in immunocompromised hosts. Here, immunocompromised and immunocompetent ferrets were inoculated with an A/H3N2 influenza virus. The ferrets were given a daily oral solution of immunosupressive drugs. After virus inoculation, animals were also treated with oseltamivir or left untreated. All immunocompromised ferrets had prolonged viral RNA presence and a high total amount of virus shedding from the throat and the nose compared to the control immunocompetent ferrets. Oseltamivir treatment of the immunocompromised ferrets reduced the total virus shedding in the nose and the throat compared to untreated immunocompromised ferrets. However, oseltamivir treatment resulted in emergence of the R292K resistance substitution in the neuraminidase protein in both immunocompromised and immunocompetent ferrets, as determined by mutation specific real-time reverse transcription polymerase chain reaction and next-generation sequencing. The immunocompromised ferret model can be used to study A/H3N2 virus shedding and is a promising model to study the efficacy of existing and novel mono and combination antiviral treatments for immunocompromised hosts.

185. A Live-Attenuated HSV-1 VC2 Vaccine Protects against HSV-2 Genital Infection in Guinea Pigs

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HSV-1 infects ~4 billion people and HSV-2 infects ~1 billion people worldwide, thus, an effective vaccine for herpes simplex viruses remains a priority. We previously published that a live-attenuated HSV-1 VC2 vaccine promotes significant protection against virulent HSV-1 or HSV-2 in a mouse model for genital herpes. To evaluate the VC2 vaccine in the guinea pig genital herpes model, guinea pigs received three vaccinations at 3 week intervals with VC2 or a HSV-2 gD subunit vaccine adjuvanted with MPL/Alum. Twenty-one days following final vaccination, animals were challenged intravaginally with $1x10^6$ PFU HSV-2. Both the gD/MPL/Alum and VC2 vaccines significantly reduced primary genital disease ($p < 0.0001$) compared to control animals. Vaccination with both vaccines significantly reduced recurrent HSV-2 lesions ($p<0.001$), the number of recurrent lesion days ($p<0.0002$), and latent viral DNA load in the DRG ($p<0.0004$). Prophylactic VC2 vaccination led to slightly reduced recurrent viral shedding between 21-56 days post challenge ($p<0.07$) compared to the control group. VC2-vaccination also induced marginally higher neutralization titers compared to gD vaccination. When the VC2 vaccine was administered as a therapeutic vaccine at 14, 28, and 42 days after challenge, the VC2 vaccine reduced the number of recurrent lesion days compared to the control group and significantly reduced recurrent shedding between 28-42 days post infection ($p<0.0004$) compared to the control and gD vaccine group during this time period. Collectively, these results show that the VC2 vaccine warrants further investigation as a potential vaccine for humans (Supported by NIAID NIH Contract # HHSN272201000008I).

187. Development and Evaluation of Lactoferrin Coated Lamivudine Nanoparticle for Effective Brain Targeting

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Central nervous system acts as a reservoir for HIV, and blood-brain-barrier (BBB) plays an important role in poor penetration of anti-HIV drug. Targeted delivery of anti-HIV drugs to the brain has many challenges, and blood-brain-barrier plays an important role in this. Lactoferrin is a glycoprotein that belongs to the transferrin (Tf) family, and facilitates transport through lactoferrin receptors by receptor mediated transcytosis. The objective of this study was to study the brain penetration enhancement of lamivudine, which are widely used in the treatment of AIDS. To overcome the challenges, we are first time reporting lactoferrin coated nanoparticle of lamivudine. Solid-lipid nanoparticles (SLN) of lamivudine were prepared using emulsification and solvent evaporation method. Lactoferrin conjugation on SLN was achieved through carbodiimide synthesis. Developed nanoparticles were further evaluated by DLS, FTIR, XRD and DSC techniques and *in vitro* release studies. The colloidal stability study was assessed in the biologically simulated environment (normal saline and serum). These coated nanoparticles are of well dispersed spherical shape with 70–94 nm size and with encapsulation efficiency of 71 ± 3.2%. Cytotoxicity studies of coated SLN showed less toxicity than lamivudine. Brain uptake studies revealed high drug penetration compared to uncoated nanoparticles and lamivudine powder drug. It is evident from the findings that lactoferrin coated nanoparticles has potential to deliver anti-HIV drug to brain than available conventional marketed formulation.

189. Development of Engineered Nanocarrier for Controlled Delivery of a Protease Inhibitor

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AIDS is a chronic, progressive syndrome, characterized by intense viral replication and profound immunosuppression, resulting in the development of life threatening opportunistic infections. HIV infection leads to deterioration of immune functions.

The objective of the present study was to develop & characterize engineered nanocarriers for controlled delivery of a protease inhibitor. Lopinavir was the drug of choice as it is an effective antiretroviral drug having specific and prominent anti-HIV action. Engineered nanocarriers targeted towards the prespecified target tissues by coupling with mannose delivers the drug in a controlled manner to the site of action. Thus it results in increased bioavailability & avoids adverse effects associated with the drug.

The uncoupled Solid Lipid Nanoparticles (SLN) were prepared by Solvent diffusion method and then coupled with mannose. Characterization studies were done by Scanning & Transmission Electron Microscopy(SEM & TEM). X-ray diffraction(XRD) & *Differential scanning calorimetry (DSC) studies were performed along with* the *in-vitro* studies followed by *in-vivo* studies on albino rats.

In-vitro & *in-vivo* studies results shows Mannose coated SLNs(MSLN) deliver their contents to macrophage rich organs and tissues, which are the reservoir of HIV. Low elimination and better distribution profile can be achieved by MSLNs. The dose of antiviral agent can be reduced due to site-specific delivery from this carrier.

Conclusively, ligand-mediated bio-disposition and cellular interaction of MSLNs, especially at target sites, would be a focal paradigm for upcoming research in the field of anti-HIV drug delivery. MSLNs have paved way for bio-stable, site-specific and ligand-mediated delivery systems with desired therapeutics.

191. *In Situ* Imaging of High Consequence Pathogens in Rodents using Fluorescent Reporter Viruses

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Nipah virus (NiV) causes encephalitis and severe respiratory illness in humans, whereas Crimean-Congo hemorrhagic fever virus (CCHFV) infection can result in severe hemorrhagic disease. Here we characterize NiV and CCHFV expressing fluorescent reporter protein ZsGreen1 (ZsG) *in vivo*. Importantly, the clinical course and outcomes of animals infected with the fluorescent viruses were comparable to those in animals infected with the non-fluorescent parental strains. Furthermore, histological examination confirmed similar cellular targets and pathological lesions in fluorescent and parental virus infections. By visualizing ZsG *in vivo*, we assessed tissue tropism, distribution patterns,

and relative infection levels of NiV and CCHFV in serially sampled and terminal animals. NiV antigen is detected in the CNS of most human cases, and occasionally in lungs and kidneys. In several terminal NiV-ZsG hamsters, robust fluorescence was observed in CNS tissues with focal lesions in the brain, and diffuse staining in the spinal cord that was associated with severe neurological signs; fluorescence was also detected in the lungs and kidneys. In humans, CCHFV mainly targets the liver, with lesser damage observed in other visceral organs. Serial and terminal CCHFV-ZsG mice demonstrated rare fluorescence in the draining lymph node early in infection, progressing to high levels of widely disseminated fluorescence, most striking in the liver, lymph nodes, and reproductive tract in terminal disease. These studies provide a unique perspective of viral distribution and support the use of fluorescent viruses to improve our understanding of pathogenesis, which will aid the development of targeted therapeutic interventions.

193. Liver Targeted Delivery of Clevudine-5′-Monophosphate Reduces Systemic Clevudine Exposure in Rats: Implications for the Treatment of HBV Infections

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Hepatitis B virus (HBV) liver infection results in either an acute infection with symptoms arising in 45 to 160 days, or in a chronic infection, which currently affects more than 350 million people worldwide. Approved treatments for chronic HBV infections, which aim to reduce HBV replication thereby limiting liver damage, include pegylated alpha interferon and nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). Clevudine, a NRTI, is a potent inhibitor of HBV replication that has been shown to reduce levels of covalently closed circular DNA (cccDNA) in the hepatitis woodchuck model. Unfortunately, phase III clinical trials (QUASH studies) were terminated after a few reports of skeletal muscle myopathy, resulting from mitochondrial dysfunction, after a long treatment period. To mitigate clevudine-associated skeletal muscle myopathy, we synthesized clevudine-5′-phosphoramidate (EIDD-2173) to 1) target clevudine-5′-monophosphate to the liver, 2) reduce systemic exposure to clevudine nucleoside, and 3) bypass thymidine kinase 2 in the phosphorylation of clevudine. Our current data shows EIDD-2173 generated higher intracellular clevudine-5′-triphosphate (CLV-TP) concentrations in rat, dog and human hepatocytes than clevudine at equimolar concentrations. In dog and human skeletal muscle cells, EIDD-2173 generated lower intracellular CLV-TP concentrations compared to clevudine at equimolar concentrations. Results from a rat pharmacokinetic and tissue distribution study indicate oral dosing with equimolar doses of EIDD-2173 or clevudine generated similar CLV-TP concentrations in liver. Furthermore, EIDD-2173 generated significantly lower concentrations of clevudine in the circulating volume and CLV-TP in skeletal muscles. These results warrant the evaluation of EIDD-2173 as a potential clinical candidate for the treatment of hepatitis B.

195. Discovery of Novel Small-Molecule Inhibitors against Chikungunya Virus: Virtual Screening, Organic Synthesis and Cell-Based Mode of Action Studies

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Chikungunya virus (CHIKV) is an arthropod-borne (arbovirus) (+)ssRNA virus belonging to the genus Alphavirus, family *Togaviridae*. CHIKV causes chikungunya fever, which is mostly characterized by fever, arthralgia and, sometimes, a maculopapular rash. Although a CHIKV infection is rarely fatal, the disease proceeds in 15-60% of infected patients into a chronic persistent disabling polyarthritis, which can severely incapacitate the patient for weeks up to several years. Due to its massive (re-)emergence and the considerable disease burden associated with infection, CHIKV has become a substantial global health threat. Neither vaccines nor antiviral drugs are available at the moment. The development of a selective and potent antiviral therapy against CHIKV is thus urgently needed.

The CHIKV genome encodes four non-structural proteins (nsP1-4), which are closely collaborating within the viral replication complex to synthesize viral RNA. The crystallized macro domain is part of the nsP3 protein and was chosen as a target for structure-based pharmacophore modelling and docking studies to identify new potential CHIKV inhibitors. The *in silico* hits were purchased and tested in a cell-based antiviral assay revealing four active compounds. A small library of analogues of the most potent hit was synthesized to highlight the essential features of the hit compound and to find molecules with improved potency. In parallel, a mechanistic study was performed with the hit compound to confirm whether the nsP3 is the molecular target of this compound or not. The compounds, their activities and preliminary results from the mechanistic studies will be presented at the conference.

196. Reverse Genetics Identifies Chikungunya Virus Nsp1 as the Target for a Novel Nucleoside Analogue

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes an acute febrile illness and severe arthralgia that may persist for months. CHIKV re-emerged in 2004 and has since spread in more than 60 countries worldwide, most recently in the Americas. The current lack of therapeutics stresses the importance of developing inhibitors with the potential to advance into the clinic. Screening of more than 70 novel selenonucleoside and carbocyclic nucleoside analogues for their activity against CHIKV, Semliki Forest virus and Sindbis virus yielded a potent compound, LJ4277, with an EC_{50} of 0.2 μM and selectivity index of >1,000. LJ4277 inhibited CHIKV in a dose-dependent manner with a more than 2.5-$\log_{10}$ reduction in infectious progeny titers at a concentration of 10 μM. Selection for LJ4277-resistant mutants identified virus variants with mutations in non-structural proteins (nsPs), mostly in nsP1 and nsP3. Reverse engineering of nsP1/3mutations into the CHIKV-LS3 infectious clone revealed that the G230R and K299E mutations in nsP1 alone or in combination with the mutation of the opal stop codon at the end of nsP3 into arginine, were responsible for resistance. These reverse-engineered LJ4277-resistant viruses were not protected against two unrelated compounds mycophenolic acid and 6-azauridine, highlighting that the nsp1 mutations are specific for resistance to LJ4277. We hypothesize that the compound could have a direct effect on alphavirus nsP1 methyltransferase activity, in addition to its indirect effect on viral replication through inhibition of cellular S-Adenosyl-L-homocysteine hydrolase. Additional biochemical assays will provide more insight into the mode of action of LJ4277.

197. Treatment of an EV-D68 Infection with Human Intravenous Immunoglobulin (Hivig) in a Respiratory and Neurological Model in AG129 Mice

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In 2014, an outbreak of Enterovirus D68 (EV-D68) spread throughout the United States and Canada. In total, 1,153 cases were confirmed in 49 states and the District of Columbia. This outbreak was temporally associated with an increase in cases of acute flaccid myelitis (AFM). During the 2014 outbreak, the CDC released recommendations for the clinical management of AFM but did not recommend the use of hIVIG. Lack of data supporting the ability of hIVIG to reduce the progression of neuroinvasive disease was the primary reason. Despite this indication, patients diagnosed with AFM in 2014 received hIVIG, steroids, and/or plasmapheresis. We evaluated hIVIG for treatment of an EV-D68 infection in separate respiratory and neurological models of disease. In a respiratory model, hIVIG did not reduce lung virus titers or histopathology of lung tissue, but reduced viremia and infiltration of the CNS. In a neurological model, hIVIG prevented paralysis and mortality when treated within 48 hours of infection. A dose of 100 mg/kg protected 100% of mice (5/5) from mortality while placebo-treated mice succumbed to infection (5/5). This provides evidence for the use of hIVIG as a treatment of an EV-D68 respiratory infection to prevent systemic viral spread. Preventing infection of the CNS could be a crucial step in preventing progression of EV-D68-induced AFM. This data indicates the use of hIVIG in EV-D68 respiratory infection is justified. Funding was provided by a grant from the National Institute of Health contract number HHSN272201000391 from the Virology Branch, DMID, NIAID, NIH Task Order A79.

198. Letermovir Resistance Analysis in a Clinical Trial of Cytomegalovirus Prophylaxis for Haematopoietic Cell Transplant Recipients

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Letermovir (LET) is a CMV DNA terminase complex inhibitor indicated for prophylaxis of cytomegalovirus (CMV) reactivation and disease in adult CMV-seropositive recipients [R+] of an allogeneic haematopoietic stem cell transplant. In a LET Phase 3 trial, CMV resistance genotyping was performed on subjects who experienced clinically significant CMV infection (csCMVI). Total DNA was isolated from plasma at the time of csCMVi, CMV genes encoding UL56p and UL89p (subunits of the terminase target) were amplified by PCR, and next-generation sequencing (NGS) was performed. Genotyping was successful for 50 of 79 LET subjects with csCMVi, including 10 subjects with detectable CMV DNA on day 1 of prophylaxis. Substitutions included many known polymorphisms with no impact on

susceptibility to LET. Fifteen novel variants (14 in UL56p, 1 in UL89p) were evaluated by recombinant phenotyping to assess their potential to confer resistance to LET. We identified 3 mutations, in viruses identified in different subjects who experienced CMV breakthrough while receiving LET, that shifted the LET EC_{50} in a cell-culture model of infection. The UL56p E237G mutation (13-fold LET EC_{50} shift) was detected in 4.1% of the NGS reads from a subject with <151 CMV DNA copies/mL, the UL56p V236M mutant (50-fold LET EC_{50} shift) was found in a subject who missed 5 of the first 10 doses of LET, and the UL56p C325W mutant (8262-fold LET EC_{50} shift) was from a subject who was CMV DNAemic on day 1. Out of 325 subjects who received LET prophylaxis, resistance was confirmed in only 3 subjects.

199. CMX521: A Nucleoside with Pan-Genotypic Activity against Norovirus

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Noroviruses belong to the *Caliciviridae* family and are classified into 7 genogroups (GI-GVII), multiple genotypes and many strains. Human noroviruses (HuNoV) are the most common cause of epidemic acute gastroenteritis worldwide (~600 million cases/year) resulting in over 4 billion US dollars in direct health care costs annually. CMX521 is a purine ribonucleoside analog which has demonstrated pan-genotypic activity against all caliciviruses tested to date. The *in vitro* EC50s of CMX521 have ranged from 0.12 to <5 micromolar against diverse caliciviruses in transformed cell lines/replicons: mouse norovirus Group V, HuNoV GI.I, HuNoV GII.4, HuNoV GII.6 and porcine sapovirus. Furthermore, CMX521 was effective against HuNoV GII.3 and GII.4 in human mucosal stem cell derived organoids. The IC50 against purified human norovirus RNA-dependent RNA-polymerase was 1-2 μM, suggesting CMX521 triphosphate is a direct-acting antiviral which inhibits the norovirus polymerase. Together, these data suggest CMX521 will be broadly effective against the diverse human noroviruses reported in contemporary outbreaks. Completed preclinical studies include evaluation of biochemical mechanism of action, *in vitro* activity against a broad panel of DNA and RNA viruses, cellular and mitochondrial toxicity, animal toxicology, rate of formation and half-life of the active triphosphate in relevant cell types, resistance passaging, enterocyte transporter identification and *in vivo* distribution studies. Orally administered CMX521 showed a dose-dependent inhibition of murine norovirus replication in mouse gastrointestinal tissues and feces. CMX521 has progressed to Phase 1 clinical development.

200. Small Molecule-Induced Degradation of Viral Targets

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We are developing small molecules antivirals that mediate proteolysis of specific viral targets through recruitment of the E3 ubiquitin ligase cereblon. Telaprevir, a covalent inhibitor that binds to the hepatitis C virus (HCV) protease active site, was used as a starting point for the design of small molecules that inhibit and induce degradation of the HCV NS3/4A protease. We have evaluated the antiviral potency of these candidates in cellular models of infection and identified several degraders that have promising antiviral activity. These were additionally shown to bind cereblon and to mediate degradation of their viral target, the HCV NS3 protein. We demonstrated that part of the antiviral activity of these molecules is contributed by targeted degradation of the protease. We are now evaluating whether the NS3-targeting degraders have superior antiviral potency against resistant protease variants that arise after treatment with the parental compound.

201. The NEDD8-Activating Enzyme Inhibitor MLN4924 Potently Inhibits Transcription from Covalently Closed Circular DNA in a Hepatitis B Virus X Protein-Dependent Manner

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BACKGROUND: Hepatitis B Virus (HBV) transcription from its template, covalently closed circular DNA (cccDNA), requires the HBV X protein (HBx)-induced degradation of the host restriction factor SMC5/6. A prerequisite for ubiquitin-dependent SMC5/6 degradation is binding of HBx to the DDB1-Cullin 4 ligase complex. Since neddylation of Cullin 4A, is required for inducing ubiquitination and subsequent degradation, we investigates whether blocking of neddylation affects HBV replication .

METHODS: We identified, MLN4924 a specific clinically applied inhibitor of the NEDD8-activating enzyme 1, for its antiviral potential. Anti-HBV activities were measured in three HBV infection models. HBx dependency was investigated using either lentiviral trans-complementation with HBx or inducible HBx-expressing stable cells.

RESULTS: MLN4924 potently reduced HBV transcription and HBsAg/HBeAg/HBcAg expressions in a dose-dependent manner in infected HepaRG^{NTCP} (IC_{50} = 60 nM) and HepG2^{NTCP} cells (IC_{50} = 500 nM). The drug acted after establishment of cccDNA and profoundly suppressed all transcripts with minimal affecting cccDNA levels. Withdrawal of MLN4924 did not lead to restoration of cccDNA transcription in HepaRG^{NTCP} cells, however reversibility was observed in HepG2^{NTCP} cells. Remarkably, HBx-mediated transcription was selectively inhibited, whereas transcription from cccDNA of an HBx-minus virions was only marginally affected indicating HBx dependency.

CONCLUSIONS: HBx promotes cccDNA transcription and our data suggest that inhibition of neddylation interferes with transcription, presumably by inhibiting HBx-mediated counteraction of transcriptional suppression. Transcription of persistent cccDNA is profoundly declined and MLN4924 treatment therefore traps cccDNA in a "transcriptional silent".

202. Strategies of Tick-Borne Encephalitis Virus to Escape from Nucleoside Analogue-Mediated Inhibition

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Tick-borne encephalitis virus (TBEV) replication was observed to be strongly inhibited by 2´-C-methyl or 4´-azido modified nucleosides, as well as by an imino-C-nucleoside BCX-4430. TBEV mutants resistant to the aforementioned nucleoside inhibitors were selected by a serial passaging of TBEV in porcine kidney stable cells in the presence of increasing concentrations of the appropriate compound. The resistance of TBEV to 2´-C-methylated nucleosides was associated with a signature mutation S603T within the active site of the viral RdRp. All-atom molecular dynamics simulations revealed that the S603T RdRp mutant repels a water molecule that coordinates the position of a metal ion cofactor as 2´-C-methylated nucleosides approach the active site. Interestingly, biological properties of the TBEV mutants were dramatically affected, which was manifested by resistance-associated loss of viral replication efficacy *in vitro* and a highly attenuated virulence phenotype in mice. TBEV resistantance to BCX-4430 was associated with two amino acid substitutions, E460D and Y453H. A reverse genetics approach (infectious-subgenomic-amplicons-based strategy) revealed, that only the mutation E460D, located within the RdRp active site, was required for a high-level resistance to BCX-4430. A molecular docking showed that the mutant D460 provides substantially lower hydrogen bonding interactions with BCX-4430 compared with the E460 wild type, which could explain the resistance mechanism of the E460D mutant to BCX-4430. Surprisingly, the subsitution Y453H was found also in TBEV mutants selected under the pressure of 4´-C-azidocytidine, however, the effect of Y453H on TBEV resistance to 4´-azido modified nucleosides is mysterious and will be addressed in our next studies.

203. The Innovative Artemisinin Derivative Artemisone Is a Potent Inhibitor of Human Cytomegalovirus Replication

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Human cytomegalovirus (HCMV) is a major cause of disease in immunocompromised individuals and the most common cause of congenital infection and neuro-sensorial disease. The expanding target populations for HCMV antiviral treatment along with the limitations of the currently available HCMV DNA polymerase inhibitors underscore the need for new antiviral agents with alternative modes of action. The anti-malarial artemisinin derivative artesunate was shown to inhibit HCMV *in vitro*, yet has demonstrated limited antiviral efficacy *in vivo*, prompting our search for more potent anti-HCMV artemisinin derivatives. Here we show that the innovative artemisinin derivative artemisone, which has been screened against malaria in human clinical studies, is a potent and non-cytotoxic inhibitor of HCMV. Artemisone exhibited an antiviral efficacy comparable to ganciclovir (EC_{50} 1.20 ± 0.46 µM) in human foreskin fibroblasts, with enhanced relative potency in lung fibroblasts and epithelial cells. Significantly, the antiviral efficacy of artemisone was consistently ≥10-fold superior to that of artesunate in all cells. Artemisone effectively inhibited both laboratory-adapted and low-passage clinical strains, as well as drug-resistant HCMV strains. By using quantitative viral kinetics and gene expression studies, we showed that artemisone is a reversible viral inhibitor, targeting an earlier phase of the viral replication cycle than ganciclovir. Importantly, artemisone most effectively inhibited HCMV infection *ex vivo* in a clinically-relevant multicellular model of integral human placental tissues maintained in organ culture. Our promising findings encourage clinical studies of artemisone as a new antiviral drug against HCMV.

204. Rational Design and Synthesis of Novel Potential Inhibitors of Zika Virus Replication

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Zika virus (ZIKV) is a *Flavivirus* transmitted to humans by *Aedes* mosquitoes which represents a global health concern. This viral infection is responsible for an acute febrile illness and it is linked to different neurological complications, such as the Guillan-Barré syndrome in adults, and microcephaly and neurological disorders in newborns to women infected during pregnancy. Despite the serious consequences associated with the infection, no therapeutic options are currently available to treat the disease.

Among the non-structural proteins responsible for the virus replication, the viral NS5 protein has an N-terminal methyltransferase (MTase) domain which plays essential roles for the methylation of the viral RNA genome 5'-end, critical for the virus life cycle, rendering it a promising target for antiviral drug design. As revealed by different crystal structures of ZIKV NS5 MTase, this protein shows the presence of a *Flavivirus*-conserved additional pocket next to the binding site of the cofactor *S*-adenosyl-methionine (SAM), which is absent in human cap-MTases and can be targeted for the identification of selective inhibitors. Starting from these observations, different novel nucleoside analogues have been rationally designed, and subsequently synthesised, in order to maintain a good predicted affinity for the Zika virus MTase SAM pocket and the ability to interact with the conserved additional cavity through substituents extending from the adenine base. The rational approach applied and the synthetic strategies optimised for the preparation of these new small molecules will be discussed, along with their activity against *Flaviviruses* MTases and antiviral effect against ZIKV replication in cellular systems.

205. Labyrinthopeptin A1 Exerts Broad-Spectrum Antiviral Activity against Dengue and Zika Virus

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Infections with flaviviruses form an increasing risk to a large part of the global human population. Recent epidemics caused by dengue virus (DENV) and the emerging Zika virus (ZIKV) highlight the importance of broad-spectrum antivirals.

We have demonstrated that the unique lantibiotic Labyrinthopeptin A1 (LabyA1) possesses antiviral activity against human immunodeficiency virus (HIV) and Herpes simplex virus (HSV) (Férir et al., 2013, PLoS ONE e64010). Here, we determine the antiviral potency of LabyA1 against DENV and ZIKV infection in several *in vitro* cellular assays. Cell viability assays and multi-parameter flow cytometry with specific viral envelope-directed antibodies show that the antiviral activity is independent of the viral serotype/strain tested or the host cell line used. The activity of LabyA1 in the flavivirus-susceptible cell lines Vero, A549 and Raji$^{DC\text{-}SIGN}$ cells ranged between 0.8-1.6 µM. Its antiviral activity was also evaluated in cell lines more relevant to the pathology of both viruses, *i.e.* the liver cell line Huh-7 for DENV (IC50: 1.7 µM), and the astroglial U87 and placental Jeg-3 cell lines for ZIKV (IC50: 0.5-2.5 µM). The antiviral activity of LabyA1 was also confirmed in monocyte-derived dendritic cells infected with different DENV serotypes using flow cytometry and qPCR. Finally, we used flow cytometry and molecular modeling to decipher LabyA1's mechanism of action. These experiments suggest that LabyA1 interferes with the interaction between the viral envelope and the cellular receptors that play a role in viral entry.

To conclude, our results suggest that LabyA1 possesses broad-spectrum antiviral activity against emerging flaviviruses.

206. Alpha-Ketoamides as Broad-Spectrum Inhibitors of Coronavirus and Enterovirus Replication

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The development of antiviral drugs targeting individual (re-)emerging viruses is often not commercially viable because of the relatively low number of patients and the self-limiting nature of the viruses. Therefore, we aim at discovering broad-spectrum antivirals with activity against larger groups of viruses. The main protease of coronaviruses and the 3C protease of enteroviruses share a similar active-site architecture and a unique requirement for glutamine in the P1 position of the substrate. Because of their unique specificity and essential role in viral polyprotein processing, these proteases are suitable targets for antiviral drugs. To obtain near-equipotent, broad-spectrum antivirals against alphacoronaviruses, betacoronaviruses, and enteroviruses, we performed structure-based design of peptidomimetic alpha-ketoamides as inibitors of main and 3C proteases. Close inspection of crystal structures of our early lead compound in complex with the target proteases HCoV-NL63 Mpro, SARS-CoV Mpro, and Coxsackievirus B3 (CVB3) 3Cpro revealed that the S2 pocket differs between these proteases: It is small and covered by a lid in the HCoV-NL63 enzyme, large and covered in SARS-CoV Mpro, and large and not covered in CVB3 3Cpro. Further, the SARS-CoV Mpro features high plasticity of the S2 site, in contrast to the two other enzymes. The best non-toxic, near-equipotent inhibitors have P2 = cyclopentylmethyl or cyclohexylmethyl and display low- or sub-micromolar EC50 values against enteroviruses, alphacoronaviruses, and betacoronaviruses in cell cultures. In Huh7 cells, one of the compounds exhibits the highest activity of any antiviral described against Middle East Respiratory Syndrome coronavirus. Named DZL08, this compound is now under preclinical development.

207. A Novel Chemotype as Hepatitis B Virus Capsid Assembly Effectors

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Chronic hepatitis B virus (HBV) infection represents a global health threat. Current FDA-approved direct acting antivirals consist of only one class of drugs, the nucleoside analogues (NAs), which do not cure HBV. Among novel antiviral strategies, targeting HBV core protein (Cp) represents a particularly attractive approach toward inhibition and infection cure of HBV. We present herein a novel chemotype as capsid assembly effectors (CAEs). The original hit with confirmed binding affinity to Cp and low micromolar antiviral activity was identified through high-throughput screening (HTS) of commercial libraries. Subsequent hit optimization through extensive structure-activity relationship (SAR) and structure-property relationship (SPR) studies involved analogue synthesis, biological assays, *in vitro* absorption, distribution, metabolism and excretion (ADME), and animal pharmacokinetics (PK). These medicinal chemistry efforts led to the identification of multiple analogues strongly binding to Cp and potently inhibiting HBV replication in nanomolar range without cytotoxicity. Two of our analogues, ZW-1066 (EC_{50} = 0.11 uM, F = 25%, CC50 >100 uM) and ZW-1042 (EC_{50} = 0.31 uM, F = 46%, CC50 >100 uM), displayed overall lead profiles superior to reported CAEs used as controls in our studies. Molecular modeling and resistance profiling provided valuable insights into their binding mode and mechanism of action.

208. A Host-Targeting Antiviral Inhibitor, STF1019, Provides Protection against Enterovirus 71 Infection in a Mouse Model

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A novel host-targeting, broad-spectrum antiviral inhibitor (STF1019) was evaluated against Enterovirus D68 (EV-D68) and Enterovirus 71 (EV-71) infection in mice. In the EV-D68 respiratory model, no mortality or weight loss is observed after intranasal challenge of 4-week-old AG129 mice. However, clinical signs after challenge with EV-D68 include viremia, decreased lung function observed by plethysmography, and elevation of virus titers and proinflammatory cytokines in the lung. Following STF1019 treatment, only modest antiviral effects were observed against EV-D68, including a potential reduction in viremia on day 1 post-infection (p.i.). In addition, cytokines were measured in lung homogenates on days 1, 3, and 5 p.i. The group treated with STF1019 at 100 or 200 mg/kg showed a significant decrease in IL-1ř on day 1 p.i., IL-1ř on day 3 p.i., and IL-6 on days 1, 3, and 5 p.i. In the EV-D68 neurological model, treatment did not provide protection from weight loss, neurological signs, or mortality after infection of 10-day-old mice. However, in the EV-71 neurological model, 100% of mice treated with 200 mg/kg STF1019 were protected from infection. STF1019 treatment also provided protection from neurological signs. In addition, mice showing early neurological signs, recovered from those signs during treatment. These results demonstrate the capability of the EV-D68 and EV-71 mouse models to evaluate antiviral therapies, and demonstrates the effectiveness of STF1019 as a potential therapy for EV-71 infection. *[Supported by Contract HHSN272201000039I from the Virology Branch, DMID, NIAID, NIH]*

209. Gamma-Non-Symmetrically-Modified d4T Triphosphates as Anti-HIV Prodrugs

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Nucleoside reverse-transcriptase inhibitors (NRTIs) are antiretroviral drugs used to treat HIV infection or AIDS. NRTIs block reverse transcriptase's enzymatic function and prevent the completion of the synthesis of the double-stranded viral DNA, thus preventing HIV from multiplying. Stavudine (d4T) is one of the NRTI drugs which approved by FDA. However, d4T is limited in its efficiency due to the necessity of intracellular phosphorylation steps by kinases. Thus, after we have introduced the first prodrug system for the intracellular delivery of nucleoside triphosphates (NTPs),[1-3] we disclose here a prodrug approach in which the gamma-phosphate of NTPs is modified by two different groups. One of these groups represents a cleavable masking group (acyloxybenzyl group) while the second group is a non-cleavable alkyl residue. The compounds showed a very high stability towards dephosphorylation as compared to d4TTP in cell extract hydrolysis studies. In antiviral assays, the compounds are potent inhibitors of HIV-1 and HIV-2 in cultures of infected thymidine kinase-deficient $CD4^+$ T-cells (CEM/TK^-). Primer extension assays using HIV's reverse transcriptase and different cellular human DNA-polymerases showed that the new compounds act as a substrate for RT. In contrast, the g-modified NTPs were found to be non-substrates for cellular DNA-polymerases beta and gamma. This opens a chance for improving the selectivity of a triphosphate derivative to act in infected but not in non-infected cells.

Reference 1-3: *Nat. Commun.* 2015, *6*, 8716; *Angew. Chem. Int. Ed.*, 2016, *55*, 5255; *Antiviral Chem. Chemother.* 2017, *25*, 69.

210. Therapeutic Treatment of Zika Virus Infection Using a Brain-Penetrating Antiviral Peptide

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Zika virus (ZIKV) is a mosquito-borne virus that is associated with neurodegenerative diseases, including Guillain–Barré syndrome and congenital Zika syndrome. As ZIKV targets the nervous system, there is an urgent need to develop therapeutic strategies that inhibit ZIKV infection in the brain. Here, we tested a brain-penetrating peptide that exhibits inhibitory activity against ZIKV and other mosquito-borne viruses. Biophysical characterization revealed that the peptide selectively targets high-curvature phospholipid membranes such as those enclosing susceptible viruses, and the peptide's composition was engineered for high *in vivo* stability. We evaluated therapeutic efficacy of the engineered peptide in a lethal ZIKV mouse model with treatment starting three days after infection. Therapeutic treatment protected against mortality and markedly reduced clinical symptoms, viral loads, and neuroinflammation, as well as mitigated microgliosis, neurodegeneration, and brain damage. In addition to controlling systemic infection, the peptide crossed the blood-brain barrier (BBB) to reduce viral loads in the brain, and protected against ZIKV-induced BBB injury. Collectively, the findings demonstrate that a brain-penetrating peptide therapeutically inhibits ZIKV infection and neurodegenerative disease, and support its potential for treating neurotropic viral infections among other possibilities.

211. Identification of a Novel Small Molecule Inhibitor of Lassa Fever Virus Entry that Targets the Viral Receptor, LAMP1

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Lassa fever virus (LFV) is a highly pathogenic, enveloped RNA virus, endemic in West Africa, with an estimated 100,000 – 300,000 cases annually. Previous studies have shown that after attachment to the cell surface, particles are endocytosed and transported to the late endosome/lysosome where acidification of the compartment promotes binding of the viral glycoprotein (GP) to its putative receptor, the cholesterol-binding, integral membrane protein LAMP1. Here, we report the identification of 3.3, a small molecule inhibitor of LFV infection, which targets the membrane distal domain (D1) of LAMP1, and competes with cholesterol for binding to D1. We show that cholesterol strongly enhances the GP-LAMP1 D1 interaction and that 3.3 blocks this interaction. We propose that GP binding to LAMP1 is dependent on the presence of cholesterol in LAMP1 D1, and that the mechanism of action of 3.3 inhibition of LFV entry is to displace this cholesterol, thereby inhibiting binding of GP to its receptor.

212. A Novel Class of Tryptophan Dendrimers Binds to the 5-Fold Vertex of Enterovirus A71 Capsid and Blocks Virus Entry

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Enterovirus A71 (EV-A71) is one of the major etiologic agents of hand, foot, and mouth disease (HFMD), which is occasionally associated with severe neurological complication in young children. So far, there are no antivirals approved to treat/prevent the infection. We reported earlier that a novel class of HIV inhibitors, the tryptophan dendrimers, unexpectedly also inhibit EV-A71 replication. The compounds are in particular highly potent (low-nanomolar/high-picomolar range) against clinical isolates. We here report on the mechanism-of-action against EV-A71 of the prototype of this compound family (MADAL_0385). By resistance selection/reverse genetics followed by cryo-EM of EV-A71 and MADAL_0385, we mapped the binding region of the prototype to the 5-fold vertex of the viral capsid and also showed that only one compound can bind on each vertex. Moreover, by means of receptor pull-down, we demonstrate that MADAL_0385 inhibits EV-A71 attachment to the host cells by blocking the interaction with at least two viral (co-)receptors: PSGL1 (P-selectin glycoprotein ligand-1) and the glycosaminoglycan heparan sulfate. Based on these observations, we speculated that the carboxyl-group of MADAL_0385 could compete with receptors binding in the positively-charged cluster of the 5-fold vertex, thereby exerting its antiviral action. In conclusion, we discovered and characterized a novel class of EV-A71 inhibitors with the most potent *in vitro* activity ever reported for enterovirus entry inhibitors and a novel mechanism of action that is very different from that of "classic" capsid binders such as pirodavir/vapendavir.

213. Towards the Development of Direct-Acting Antivirals for the Treatment of Human Parechoviruses Infections

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Parechoviruses (HPeV, *Picornaviridae*) are neglected human pathogens causing sepsis-like illness and life-threatening neurological complications in infants. There are no antivirals available for the treatment of HPeV and there is a lack of optimized assays to test the antiviral potential of small molecules. In order to establish a convenient antiviral assay for HPeV1-3 infection, we first selected a cell line in which the virus replicates efficiently resulting in good CPE. To that end BGM cells (an african green monkey kidney line) were selected. Next, the kinetics of replication of HPeV1 and HPev3 were analysed in these cells: intracellular viral RNA peaked at 6h post infection for HPeV1 and at 12h p.i. for HPeV3, but by at 3 days p.i., the extracellular genome copy numbers of both viruses were comparable. We next studied whether a panel of molecules that were earlier shown to inhibit entero/picornavirus replication, inhibit HPeV1/3 replication Favipiravir (T-705), as well as nucleoside analogs 7-deaza-2'*C*-methyladenosine (7DMA), 2C-methylcytidine (2CMC), 2C-methylguanidine(2CMG), 2C-methyladenosine (2CMA) inhibited HPeV1/3, with 2CMC being the most effective (EC90 10,5 μM). Enterovirus-specific inhibitors (pleconaril, rupintrivir, SMK_0213) were devoid of any activity against HPeV.

We next performed a screening of the NCI library of ~2000 small molecules. Following confirmatory assays on hit-compounds, ten molecules were selected that resulted in 1-1.5 log 10 inhibition of viral RNA-yield. The particular characteristics of the antiviral activity of one of there is currently being further studied. In conclusion, we established an antiviral assay platform that allows medium-throughput screening for HPeV iinhibitors.

214. Role of Nonstructural Protein 1 in the Replication Cycle of Dengue Virus

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Dengue virus (DENV) is the most prevalent mosquito-borne viral pathogen worldwide, estimated to infect around 400 million people annually. Despite the profound socio-economic impact of DENV-associated diseases, no antiviral therapy is available and many steps of the viral replication cycle are poorly characterized. Nonstructural protein 1 (NS1) of DENV is essential for viral RNA replication; however the underlying molecular mechanism remains elusive. We have previously reported a comprehensive panel of NS1 mutants completely abrogating DENV replication. Here, we further characterize these mutants by using replication assays, co-immunoprecipitations, electron microscopy and structure analysis. We report a novel interaction between NS1 and the NS4A-2K-NS4B precursor polyprotein and identify NS1 residues Gly161 and W168 as critical determinants for this interaction. Alanine substitutions affecting these sites completely abrogated precursor protein binding and abolished RNA replication, but did not affect NS1 secretion. By using electron microscopy, we obtained preliminary evidence suggesting that the interaction between NS1 and the NS4A-2K-NS4B precursor is not necessary for the formation of membranous DENV replication organelle. Our results provide novel insights into contribution of NS1 to DENV replication and argue for a functional role of the NS4A-2K-4B precursor in the viral life cycle. Further, the study suggests that the interface between NS1 and the NS4A-2K-NS4B precursor might be a promising target for the development of DENV-specific antiviral therapy.

215. Synthesis and Evaluation of Mono-, Di-, and Tetra-acylated Prodrugs of IHVR-19029

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We discovered a novel compound, N-alkyl-ureanyl-deoxynojirimycin, or IHVR-19029, that has been demonstrated to significantly protect mice from lethal infections of the Marburg and the Ebola viruses when administrated via injection, by inhibition of the host endoplasmic reticulum (ER) ř-glucosidases I and II. However, the major obstacles toward the development of oral available IHVR-19029 are their short plasma half-life, low oral bioavailability, and inhibition of carbohydrate-metabolizing gut glucosidases, which results in osmotic diarrhea side effect. To overcome these problems, several types of prodrugs were designed and synthesized, including ester, carbonate, and amino acid prodrugs. Concise synthetic strategies were developed to prepare the fully protected tetra-acyl, or partially 2,3-di-acyl, 2- or 3- mono-acyl IHVR-19029 prodrugs. As enzymatic assays showed, all the prodrugs lost the ability to inhibit ER α-glucosidases I and II, suggesting that these ester prodrugs would have reduced activity against GI α-glucosidases, and potentially overcome the off-target effects of the parent compound. In a pilot cell based assay, these prodrugs remained antiviral activities against dengue virus infection, demonstrating efficient release of parent IHVR-19029 from these prodrugs inside cells after absorption. Other *in vitro* ADME profiling studies indicated that many of these prodrugs are predicted to be stable in the GI and have rapid biological conversion within the circulation as well as inside the cells. These results suggested that the prodrug approach is likely leading to the improved oral bioavailability, toxicity, and antiviral efficacy against multiple enveloped viruses.

216. *In Vivo* Replication of Human Norovirus in Zebrafish Larvae

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The lack of robust small animal models for a human norovirus (HuNoV) infection has significantly hindered the development of effective therapeutic strategies. Only recently, the first *in vitro* culture systems to study HuNoV became available. Noroviruses are the most common cause of foodborne illness, having a societal cost of $60 billion and 219,000 deaths/year. We have identified that HuNoV of multiple genotypes are able to robustly replicate in zebrafish (*Danio rerio*), a small optically transparent animal, suitable for live imaging and whole-organism pathology.

HuNoV infection in 3-day-old zebrafish larvae peaks at day 2 post infection and is detectable for at least 5 days. Treatment with a specific antiviral reduced the HuNoV viral RNA load by >2 $\log_{10}$, further demonstrating that the virus can replicate efficiently in this model. Additionally, we detected non-structural norovirus proteins by western blot and structural antigens via an enzyme immunoassay. The organs/tissues in which norovirus replicates were identified by immunohistochemistry. The downregulation of a *fucosyltransferase* gene limited the replication of HuNoV in zebrafish, thus highlighting a key common feature with this infection in humans. Overall, this model is a major step forward in the development of models to study human noroviruses and this study is the first evidence that human noroviruses can replicate robustly *in vivo* in a small laboratory animal. Ultimately, this will help us understand norovirus biology and bring us closer to the discovery and development of antiviral drugs.

217. Identification of Small Chemical Compounds Blocking Tegumentation and Assembly of Herpes Simplex Virus

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Infections with the herpes simplex viruses (HSV-1; HSV-2) cause significant global disease burden, particularly in immunocompromised patients, with life threatening infections of newborns, long-term disabling encephalitis and eye infections. Currently, there are no licensed vaccines, but there are drugs used in the clinic that target viral DNA replication. The risk of selecting resistant viral mutants could be reduced by complementing the existing treatment with novel compounds targeting capsid assembly or tegumentation and thus virion assembly.

We used our reporter strain HSV1(17$^+$)Lox-GFP in a phenotypic screen and identified 61 of about 19,000 compounds that reduced production of infectious virus. Of those, 27 inhibited plaque formation in Vero, HeLa and the human keratinocyte HaCaT cell lines at IC_{50} ranging from 1.4 to 20 µM; while MTT and ATP assays showed CC_{50} from 15 to more than 200 µM. For some of them, the selectivity indices were higher than for acyclovir, the current gold standard for clinical HSV-1 and HSV-2 therapy. Further experiments with our dual color strain HSV1(17$^+$)Lox-CheVP26-VP11/12GFP provided first insights into which steps of the viral life cycle were targeted. While some compounds inhibited nuclear capsid formation or capsid egress into the cytoplasm, others blocked the binding of the C-terminal domain of pUL36, the large essential tegument protein, to nuclear and cytoplasmic capsids.

In summary, we have identified 3 compounds that inhibited HSV-1 and HSV-2 infection as potently as acyclovir, and which we will examine further to identify their molecular viral or host target.

219. The Therapeutic Human Bispecific Neutralizing Antibody FIT-1 Treats Congenital Zika Disease

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In order to facilitate the discovery of potential interventions for congenital exposure to Zika virus (ZIKV), we have developed a mouse model for use in antiviral studies. Congenitally exposed AG129 mouse pups are significantly smaller, have reduced head lengths and have detectable viral RNA titers at various times before and after birth. A transient hearing loss was also observed in some, but not all, pups exposed to ZIKV in utero. In order to determine the effect of antiviral treatment on disease in this congenital exposure model, we treated pregnant dams with 45 mg/kg of FIT-1, a ZIKV-specific human bispecific neutralizing antibody. Dams were infected on day 7 of pregnancy and treatment was administered 24 or 72 h post-virus challenge, A cohort of dams were necropsied 11 days after virus challenge (day 18 of pregnancy) to determine the effect of treatment on fetal size and viral load. A significant improvement in dam survival was observed after treatment with FIT-1. Treatment also resulted in a significant reduction in viral RNA in the placenta and fetus, as well as in maternal spleen and brain samples. There was also a trend toward increased pup size, placental weight. The data also suggested earlier treatment was more effective, although treatment initiated 72 h after virus challenge was highly effective in improving disease outcomes in congenitally exposed pups. Further studies testing FIT-1 for the treatment of congenital infection are warranted. [Supported in part by HHSN272201000039I from the Virology Branch, NIAID, NIH]

220. Differential Antiviral Activities of RSV Inhibitors in Human Airway Epithelium

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We report the use of reconstituted 3D-human airway epithelium cells of bronchial origin (HuAEC) in an air-liquid interface to study respiratory syncytial virus (RSV) infection and to assess the efficacy of RSV inhibitors in (pre-)clinical development. RSV-A replicates efficiently in HuAEC and viral RNA is shed for weeks after infection. RSV infection reduces the ciliary beat frequency of the ciliated cells as of 4 days post infection, with complete ciliary dyskinesia observed by day 10. Treatment with RSV fusion inhibitors resulted in an antiviral effect only when added at the time of infection. In contrast, the use of replication inhibitors (both nucleoside and non-nucleosides) elicited a marked antiviral effect even when start of treatment was delayed until one or three days after infection. Levels of the inflammation marker RANTES (mRNA) increased ~200-fold in infected-untreated cultures (at three weeks post infection), but levels were comparable to those of uninfected cultures in the presence of PC-876, a RSV-replication inhibitor, demonstrating that an efficient antiviral treatment inhibits virus induced inflammation in this model. Overall, HuAEC offer a robust and physiologically relevant model to study RSV replication and to assess the efficacy of antiviral compounds.

221. ADAR1 is a Regulator of Innate and Antiviral Immune Function in HCV Infection.

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The hepatitis C virus (HCV) is a globally prevalent infectious pathogen. As many as 80% of people infected with HCV do not control the virus and develop a chronic infection. Response to interferon (IFN) therapy is widely variable in chronic HCV infected patients, suggesting that HCV has evolved mechanisms to suppress and evade innate immunity responsible for its control and elimination. ADAR1 is a relevant factor in the regulation of the innate immune response. Here, we describe ADAR1 role as a regulator of innate and antiviral immune function in HCV infection, both *in vitro* and *in vivo*. Polymorphisms within ADAR1 gene (rs2229857 and rs1127326 were found significantly associated to poor clinical outcome to HCV therapy (p=0.012 and p=0.014) and advanced liver fibrosis fibrosis (p=0.0049 and p=0.0036) in a cohort of 155 HCV and HIV-1 coinfected patients. Moreover, ADAR1 knockdown in primary macrophages and Huh7 hepatoma led to significant increase in *IFNB1* expression (7.5-fold and 4-fold, respectively) and CXCL10 production (1000-fold and 3-fold, respectively) compared to mock-transfected cells. Knockdown of *ADAR1* enhanced expression of RNA sensors RIG-I and MDA5, phosphorylation of STAT1 and expression and phosphorylation of IRF7, indicative of innate immune activation. In addition, susceptibility to HCV infection was significantly increased in *ADAR1* knockdown cells (3.5-fold, p=0.034). Overall, our results demonstrate that ADAR1 regulates innate immune signaling and is an important contributor to the outcome of the HCV virus–host interaction. ADAR1 is a potential target to boost antiviral immune response in HCV infection.

222. A Novel Benzamide Compound that Distinctly Modulates HBV Capsid Assembly

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Hepatitis B virus (HBV) core protein is a small protein with 183 amino acid residues and plays multiple roles in the viral replication cycle. One of its best characterized functions is to assemble the pre-genomic (pg) RNA-viral DNA polymerase complex to form nucleocapsids where HBV DNA synthesis takes place. During the last decade, more than six chemotypes of HBV core protein allosteric modulators (CpAMs) have been reported. These compounds bind to a hydrophobic pocket, designated as HAP pocket, at the dimer-dimer interface and induce allosteric conformational changes in core protein subunits. While heteroaryldihydropyrimidines (HAPs), such as Bay 41-4109 and GLS-4, misdirect core protein subunits to form non-capsid polymers, all other chemotypes of CpAMs, including sulfamoylbenzamides (SBAs) benzamides (BAs) and phenylpropenamides (PPAs), induce the formation of empty capsids with distinct structure changes and faster migration mobility in native agarose gel electrophoresis. Through a high throughput screening of our in-house compound library, we recently identified a novel benzamide derivative, designated as BA-53038B, which induced the formation of empty capsids with the similar electrophoresis mobility of wild-type capsids. Genetic and drug resistant analyses indicated that BA-53038B most likely bound to HAP pocket, but obviously modulated HBV capsid assembly in a distinct mechanism. Further comparative structural analyses of

the capsids formed in cells treated with BA-53038B and other BA compounds should shed light on the mechanism underlying CpAM modulation of capsid assembly pathways to favor the assembly of empty capsids, but not pgRNA-containing nucleocapsids.

223. Hepatitis B Virus Replication Inhibition by *N*-hydroxypyridinediones and N-hydroxyisoquinolinediones in Culture through Inhibition of the Viral Ribonuclease H

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We recently developed a screening paradigm capable of selectively identifying and evaluating inhibitors of the Hepatitis B virus (HBV) ribonuclease H (RNaseH), which is the only viral enzyme not targeted by current anti-HBV therapies. Inhibiting the HBV RNaseH blocks synthesis of the (+) polarity DNA strand, causes early termination of (-) polarity DNA synthesis, and causes accumulation of RNA:DNA heteroduplexes. We previously reported inhibition of HBV replication by 11 of 15 N-hydroxyisoquinolinediones (HID) and 1 of 2 N-hydroxypyridinediones (HPD) in hepatoma cells. Here, we report the results from our ongoing efforts to develop more potent anti-HBV RNaseH inhibitors in the HID/HPD compounds classes. We synthesized and screened 26 new compounds, 7 HIDs and 19 HPDs, for preferential suppression of (+) polarity DNA in HBV replicating cells. Only 3 of 7 newly synthesized HIDs inhibited HBV replication and the therapeutic indexes (TI = CC_{50}/EC_{50}) did not improve over what we previously reported. However, 14 of 19 HPDs inhibited HBV replication with EC_{50}s ranging from low-to-mid nM to 4 μM. Cellular cytotoxicity was evaluated by three assays and CC_{50}s ranged from >100 to 15 μM. The best compounds have a TI of >300, which is a significant improvement over the primary HPD hit. These studies indicate that anti-RNaseH drug discovery is advancing and the HPD compound class holds significant potential for antiviral development. Our future studies will be informed by our growing structure activity relationships.

224. Mimicking the HRB Region of RSV F Protein as Antiviral Strategy

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Respiratory Syncytial Virus (RSV), part of to the Paramyxoviridae family, is an enveloped, negative-sense, single-stranded RNA virus and it is the principal etiological cause of LRTIs (Low Respiratory Tract Infections) worldwide. It causes bronchiolitis that may lead to life-threatening pneumonia and it is involved in the development of long-term respiratory diseases, such as asthma in adults. Currently, there is no effective small-molecules antiviral on the market for the treatment or prevention of this viral infection.

In 1996, synthetic peptides with the helical structure of the heptad repeat B (HRB) region of the F protein were reported to be potent inhibitors of the RSV viral fusion in cell-based assays. Starting from the sequence of HRB showing antiviral activity and based on the model of the fusion process, the HRB ^{488}FDASISQVN^{496} fragment was selected as template for the rational design of ř-helix mimics, able to inhibit the protein-protein interactions between the trimeric inner coiled-coil and the external ř-helixes.

Several chemoinformatic techniques were used to generate and evaluate a focussed virtual library of compounds designed to mimic the hydrophobic backbones in positions *i, i+4, i+7* of the ^{488}FDASISQVN^{496} fragment. The generated library was used for a structure-based virtual screening on the X-ray structure of the post-fusion F protein. The most promising compounds were synthesised and evaluated for antiviral activity in a virus-cell-based assay and the results will be discussed at the conference.

225. Pharmacological Inhibition of CDK4/6 Enhances Antiviral and Cytotoxic Activity of Antimetabolites

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Cell cycle control and HIV-1 susceptibility are linked by a CDK-dependent phosphorylation of SAMHD1, a key player in maintaining a balanced intracellular dNTP pool and critical for viral replication. Selective CDK4/6 inhibitors impede SAMHD1 phosphorylation, activating SAMHD1 function and therefore restricting HIV-1 replication. HIV-2 Vpx degradation of SAMHD1 modulates nucleoside analogue efficacy. Thus, it is plausible that pharmacological activation of SAMHD1 could exert a similar effect. Here, we evaluate the capacity of palbociclib, a highly selective CDK4/6 inhibitor, to modify antiviral activity of antimetabolites. Anti-HIV and cytotoxic activity of pemetrexed was evaluated alone or in combination with palbociclib in primary and established cell lines. Pemetrexed inhibited HIV replication in a dose-dependent manner although with limited potency (EC_{50}=0.1 μM). Combination of pemetrexed with increasing concentrations of palbociclib enhanced the antiviral potency and cytotoxicity of the antimetabolite (6-fold change, 92% inhibition), indicating strong synergy (Combination Index=0,0049-0,404). Interestingly, pemetrexed and palbociclib antiviral activity was lost in the absence of SAMHD1, as well as the synergistic effect observed in drug combinations. These results suggest that palbociclib-mediated enhancement of antimetabolite antiviral activity is dependent on the regulation of SAMHD1 phosphorylation by CDK4/6. Analysis of cell cycle profile and protein expression of drug-treated cells confirmed that CDK4/6 control SAMHD1 phosphorylation but also demonstrates its correlation with antiviral activity. In summary, modulation of SAMHD1 phosphorylation by highly selective CDK4/6 inhibitors, have the potential to improve antimetabolite-based antiviral and anti-cancer therapies and pave the way to the use of treatment combinations that fine tune drug efficacy.

226. Chemical Genetic Studies Revealed a Novel Mechanism of Innate Immune Evasion by Yellow Fever Virus NS4B That Can Be Targeted by an Antiviral Compound

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We reported previously a benzodiazepine compound BDAA that was discovered from HTS, and specifically inhibited yellow fever virus (YFV) *in vitro* and *in vivo* in hamsters (Guo, F., et al, J. Virol. 2016). Drug-resistant virus selection and reverse genetics studies suggested that a three residue motif centering at P219 of NS4B protein may define the interaction between BDAA and YFV. In our efforts to elucidate the mode of action, we found that BDAA also specifically enhances YFV RNA-induced cytokine response. Specifically, RNAseq analysis revealed that after the onset of YFV RNA replication, BDAA treatment of the infected cells, for as short as 2 hours, significantly enhanced the levels of mRNAs specifying a broad range of cytokines/chemokines and ISGs. Moreover, using CRISPR/Cas9 knockout technology, we demonstrated that MAVS (or IPS-1), the adaptor of RNA sensor RIG-I and MAD5, is essential for BDAA enhancement of YFV-inducted cytokine response, but not for inhibition of YFV replication. However, studies with a panel of BDAA-resistant YFV indicate that BDAA enhancement of cytokine response does rely on its specific interaction with NS4B. Our findings support the hypothesis that BDAA interaction with NS4B protein may impair the integrity of YFV replication complex, which inhibits viral RNA replication and promotes viral RNA releasing from replication complex and consequentially activates RIG-I and/or MDA5. Taken together, our studies indicate that targeting of YFV NS4B protein with BDAA and its analogues simultaneously disrupt NS4B's functions in viral RNA replication and evasion of host cellular innate immune response.

227. Antiviral and Immunological Adjuvant Efficacy of Synthetic RNA as a RIG-I Agonist against Influenza A Virus Infection

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Retinoic acid-inducible gene I (RIG-I) recognizes double-stranded RNAs (dsRNAs) containing two or three 5ř phosphates. A few reports of 5ř-tirphosphate-independent RIG-I agonists have emerged, but little is known about the molecular principles underlying their recognition. Recently, it was suggested that the bent duplex RNA from the influenza A panhandle promoter activates RIG-I even in the absence of a 5ř-triphosphate moiety. We observed

that non-canonical synthetic RNA oligonucleotides containing G-U wobble base pairs that form a bent helix can exert RIG-I-mediated antiviral effects in a sequence- and site-dependent manner. We present synthetic RNAs that have been systematically modified to enhance their efficacy and outline the basic principles for engineering RIG-I agonists applicable to immunotherapy. Transfection of the optimized RIG-I agonist RNA, named CBS-13-BPS, potently suppressed viral protein expression, resulting in no expression of NP derived from A/Puerto Rico/8/34 (PR8) at a concentration of 1 nM. Its antiviral efficacy was comparably reproduced against oseltamivir-resistant PR8 virus. Notably, intranasal immunization of mice with inactivated PR8 (vaccine) and CBS-13-BPS (adjuvant) afforded complete protection against mouse-adapted PR8 (maPR8) infection, while only 40% of mice vaccinated with vaccine alone survived maPR8 challenge. We suggest that non-canonical synthetic RNA without 5′-triphophate plays a role as a RIG-I agonist; it efficiently suppresses influenza A virus replication within target cells and stimulates antigen-specific IgG response *in vivo* as an intranasal vaccine adjuvant.

228. Simultaneous and Consecutive Infections with Different Herpesviruses among Immunocompromised Patients

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BACKGROUND: Herpesviruses, in particular HCMV, HSV-1, HSV-2, and VZV but also HHV-6, represent a significant cause of morbidity and mortality in immunocompromised hosts, mainly following transplantation. Our goal was to determine the emergence of drug-resistance among immunocompromised patients (refractory to antiviral therapy), who developed infections either simultaneously or consecutively with more than one of these herpesviruses.

METHODS: Genotyping of the thymidine kinase (TK) (HSV and VZV), protein kinase (PK) (HCMV, HHV-6) and DNA polymerase (DP) (HSV, VZV, HCMV and HHV-6) genes was performed in several samples recovered from 25 immunosuppressed patients that had refractory infections caused by one of these viruses under the RegaVir translational research platform for typing herpesvirus drug-resistance.

RESULTS: The most common infections with different herpesviruses in a single patient were due to HSV-1 and HCMV infections (20 out of 25 patients), with two cases of simultaneous infections. Consecutive infections with HSV-2/HSV-1, HHV-6/HCMV, HSV-1/VZV, and VZV/HCMV/HHV-6 were found in single patients. Only 6 patients did not develop herpesvirus drug-resistant during their follow-up, while a resistant genotype was identified for a single herpesvirus (12 patients) or two different herpesviruses (6 patients). During the surveillance of drug-resistance emergence, different genotypic profiles for a given virus were identified in individual patients (n=8). Further, TK/DP HSV-1 (3 patients) and PK/DP HCMV (3 patients) double mutants were identified, including a multi-drug resistant HCMV genotype.

CONCLUSIONS: Important changes in herpesvirus infections in immunocompromised infected with more than one herpesviruses were found with emergence of an important number of drug-resistant viruses.

229. Identification of a Novel Immunomodulatory Pathway as Candidate Target against Zika Virus

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Zika virus (ZIKV) is a mosquito-borne flavivirus of the Flaviviridae family that was first identified in Uganda in 1947. Since its emergence in Brazil in May 2015, ZIKV has raised global concern due to its association with a significant rise in the number of infants born with microcephaly and neurological disorders. So far, no vaccines or antiviral therapies have been developed against this flavivirus. In order to address this issue, the present study investigates the targeting of host functions essential for viral replication. We began by analyzing a list of host target candidates through an RNAseq screening from which we selected AhR for further characterization. AhR is a ligand-dependent transcription factor that can be activated by a broad range of molecules present in the environment and commensal diet, microbiota, and metabolism. Our results showed for the first time that AhR is specifically activated upon ZIKV infection *in vitro*. Moreover, pharmacological modulation of AhR using agonist and antagonist molecules, increases and decreases ZIKV extracellular production in human liver, murine astrocites and human neuroprogenitor cell cultures, respectively. Also, our study reveals that this observation is partially independent of the IFN-I and provides a possible mechanism of action for the inhibitor CH-223191 in the context of ZIKV infection. Further experiments *in vivo* will confirm the role of AhR as a regulator of the immune response to ZIKV, and its potential use as a novel target against this pathogen. Altogether, our results will guide further development of broad spectrum antiviral molecules.

230. Neurodevelopmental Sequelae Following Congenital CMV: Role of Virus-Induced Inflammation

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Human cytomegalovirus (HCMV) is the most common infectious agent transmitted to the developing fetus and a major cause of long-term neurodevelopmental sequelae in infants infected in-utero. Regardless of the clinical findings in the newborn period, long-term sequelae that follow congenital HCMV infection are almost exclusively associated with central nervous system (CNS) damage. Although the natural history of this congenital infection has been extensively studied for over 4 decades, the mechanism(s) of disease leading to CNS damage remains incompletely defined. Autopsy specimens and in-vitro model systems have suggested that HCMV can lytically infect neural stem cells and neuroprogenitor cells and alter developmental programs in these cell types. However, clinical observations and findings from imaging studies argue that lytic infection resulting in CNS damage is an unlikely explanation for the majority of infants with neurodevelopmental sequelae associated with congenital HCMV infection. More recently, studies in small animal models, as well as analysis of CNS specimens from autopsied cases of infected fetuses have provided evidence suggesting that host inflammatory responses in CNS could contribute to the altered neurodevelopment that is observed in congenitally infected infants. We have exploited the neurodevelopmental status of newborn mice and the closely related murine cytomegalovirus (MCMV) to define mechanisms of CNS disease following infection during neurodevelopment. Our studies have clearly demonstrated that host inflammatory responses to MCMV infection are responsible for altered neurodevelopment of mice that are infected during CNS development. These findings raise the possibility that antiviral therapy coupled with agents that limit inflammatory responses in the CNS could further improve the long-term outcome of infants with congenital HCMV infections.

231. Mutagenesis of the Human Genome to Study Virus Entry

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Our laboratory applies genetics in human cells to generate overviews of genes that play a role in phenotypes of interest. To achieve this we use haploid human cells in combination with insertional mutagenesis as a genetic model system. We have applied this approach to identify host factors that are used by viruses. This resulted in the identification of new cell surface receptors for cell entry, intracellular receptors for Ebola- and Lassa virus as well as new host factors that modulate endosome escape of picornaviruses.

232. A Primer on Cryo-EM and Image Reconstruction for Antiviral Drug Development

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In electron cryo microscopy (cryoEM), a sample in aqueous solution is first vitrified by rapid plunging into liquid ethane, then it is placed in a specialized transmission electron microscope and then imaged in this "frozen-hydrated" state. The cool temperature prevents dehydration in the vacuum of the electron microscope, and also helps to protect the sample from beam damage. The images are recorded on very sensitive direct electron detectors as two-dimensional projections of the original three-dimensional object, akin to the result of an X-ray picture. We merge these images together to generate an average three-dimensional representation of the original object, which in the best case can be used to model the atomic coordinates of the object. Thus eliminating the need for crystallization. The objects are typically in the size range of 5-200 nm. I will give examples from antiviral drug development, where we have discovered new pockets or interactions to target, and where we have resolved the position of a bound small inhibitory molecule in a viral capsid to explain the mode of action.

233. Transcriptional Profiling of HIV Latency

Angela Ciuffi[1]
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Despite effective treatment, HIV can persist in latent reservoirs, which represent a major obstacle towards HIV eradication. Targeting and reactivating latent cells is challenging due to the heterogeneous nature of HIV infected cells. We used a primary model of HIV latency and single-cell RNA sequencing (scRNA-seq) to characterize transcriptional heterogeneity during HIV latency and reactivation, and to understand transcriptional programs leading to successful reactivation of HIV expression. Transcriptional analysis of 224 single cells identified two CD4+ T cell populations that might reflect two distinct resting cellular states, and displaying a different potential for cell stimulation and HIV reactivation. Our results identified a 134-gene specific cellular signature, associated with success of HIV reactivation, hence marking the inducible cell. These data should provide a valuable tool to facilitate the identification of successful latency reversing agents and help designing targeted strategies for purging the HIV latency reservoirs.

234. HBc and CAMs: a Tale of "Swiss-Knife" Protein and Antivirals

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As standard of care treatments of chronic hepatitis B virus (HBV) infections remain suboptimal, novel drugs targeting novel step of the HBV life-cycle are currently being developed. Among those are the core/capsid assembly modulators (CAMs), which primarily disrupt the formation of the viral nucleocapsid and subsequent capsid-associated reverse-transcription of the pre-genomic RNA into virion-containing rcDNA. However, the core/capsid/HBc protein, besides its structural role, is also endowed with regulatory functions in particular in the nucleus of infected cells. If HBc is mainly an RNA-binding protein, it can also interact with double-stranded DNA. Hence, HBc can bind to the cccDNA (covalently-closed-circular DNA), which is the episomal template of viral transcription in the nucleus, and might regulate its stability or/and activity. HBc is also likely involved in viral and host-cell RNA metabolism. Post-translational modification, including phosphorylation, is involved in the regulation of the various functions of this protein. Due to the multiple functions of HBc, CAMs are expected to inhibit more than nucleocapsid assembly. This lecture will briefly touch upon the biology of HBc in the HBV life-cycle as well as mechanisms of action of CAMs, before summarizing current investigational and clinical efforts to develop this novel class of anti-HBV drugs.

235. Ebola Treatment: Working in the Dark under the Spotlight

Sir Michael Jacobs[1]
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This presentation will describe the development of medical countermeasures against Ebola over the course of the West Africa outbreak, their experimental use during the outbreak, clinical studies performed and subsequent progress. It will consider how the global medical community should prepare to use medical countermeasures in future outbreaks.

236. Treatment of Congenital CMV Infection: New Populations, New Regimens, and New Drugs?

David Kimberlin[1]
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Congenital cytomegalovirus (CMV) infection can result in clinically apparent (symptomatic) disease at birth, or in clinically inapparent (asymptomatic) infection. Diagnosis of neonates with symptomatic CMV disease generally is relatively straightforward, since those patients have clinical manifestations that drive the diagnostic approach to them. In contrast, diagnosis of asymptomatic congenital CMV infection can only be achieved through broad-based screening programs since those patients are completely normal in appearance. Data on the treatment of congenital CMV infection are only available for babies with symptomatic disease, with treatment started in the first month of life in all of the studies completed to date. A previous randomized controlled trial conducted in the 1990s by the Collaborative Antiviral Study Group (CASG) demonstrated that administration of six weeks of intravenous ganciclovir to babies with symptomatic congenital CMV disease protects against hearing deterioration over the first six months of life. The dose of oral valganciclovir that produces similar blood concentrations of ganciclovir as does intravenous ganciclovir was identified in a subsequent CASG trial in the 2000s. In the 2010s the CASG completed a multicenter, multinational, randomized placebo-controlled trial that determined that six months of valganciclovir therapy is superior to six weeks of valganciclovir in protecting hearing deterioration and improving developmental outcomes at 12 and 24 months. A new CASG study is assessing valganciclovir therapy in infants with asymptomatic congenital CMV infection. An ongoing CASG study is assessing valganciclovir started beyond the first month of life. As additional antiviral drugs with CMV activity are developed, clinical investigations of their use in combination with valganciclovir in infants with congenital CMV infection are warranted.

237. Letermovir: Current State of the Art

Randi Leavitt[1]
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There is an unmet need for safe and efficacious agents for CMV prophylaxis in hematopoietic stem cell transplant (HSCT) recipients. Until recently, all anti-CMV drugs were nucleoside analogs with significant toxicities. Two approaches are used to prevent CMV disease in HSCT recipients. The first is prophylaxis, where antivirals are initiated prior to viremia in order to prevent any level of viremia. Alternatively, pre-emptive therapy (PET) is initiated (usually with ganciclovir or valganciclovir) once viremia is detected, but this approach can be problematic in HSCT patients, particularly prior to engraftment, since these drugs are associated with significant toxicities, including myelotoxicity. Prophylaxis is a preferable strategy since PET is started after viremia is detected and any level of viremia is associated with an increased risk of overall mortality. A safe and efficacious prophylactic agent is of major public health interest and should provide significant benefit to the HSCT population.

Letermovir inhibits cytomegalovirus (CMV) through a novel mechanism involving the viral terminase complex which is required for DNA cleavage into unit length genomes and packaging into procapsids. Letermovir has potent anti CMV activity *in vitro* and *in vivo*. There is no cross-resistance with drugs currently used in the treatment of CMV as drug resistance to letermovir has been mapped to UL56 whereas resistance to other anti CMV agents, which are all DNA polymerase inhibitors, map to UL54 and UL97.

In a pivotal phase 3 study in CMV seropositive recipients of an allogenic HSCT, letermovir was highly efficacious in preventing clinically significant CMV infection through week 24 post-transplant, demonstrating ~40% relative reduction compared to placebo. Efficacy was demonstrated across a broad range of subgroups.

Mortality was decreased by~30% compared to placebo at week 24 and ~14% compared to placebo at week 48.

Letermovir was generally well tolerated with an adverse event profile similar to that of placebo with no evidence of myelotoxicity.

These data demonstrate that letermovir prophylaxis provides meaningful benefit after HSCT.

238. Getting to the Root of Epidemic Spread: An Evolutionary Perspective on Pathogen Emergence

Philippe Lemey [1]

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Evolutionary analyses of genetic data have played a prominent role in reconstructing the epidemic history of viruses. They have shed light on the origins, evolution and transmission dynamics of different viruses that have a longstanding circulation history in humans (e.g. HIV, HCV, and Influenza). The ability to rapidly generate genomic data through next-generation sequencing technologies is now also opening up such opportunities for viral outbreaks on smaller time-scales (e.g. Ebola and Zika). A critical challenge, however, lies in developing methods that can efficiently extract epidemiological information from genomic data.

Here, I will introduce key concepts of molecular epidemiology by demonstrating how they offer a genomic window into the past of HIV-1. Next, the opportunities and challenges of applying phylodynamic inference methods in outbreaks scenarios will be highlighted. I will discuss the application of Bayesian phylogenetic and phylogeographic inference techniques to reconstruct the patterns and drivers of viral spread during the West African Ebola epidemic. Finally, I will illustrate how recent genomic analyses of Lassa fever have contributed to the response to largest outbreak ever reported in Nigeria.

239. Identification of a Capsid-Binding Protein that Recognizes the Nuclear HIV Capsid to Promote CGAS-Mediated Innate Immune Activation in Dendritic Cells

Nicolas Manel[1]

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The innate immune DNA sensor, cyclic GMP-AMP synthase (cGAS), is essential for induction of an innate immune response to infection by HIV-2 or HIV-1 with Vpx in human monocyte-derived dendritic cells (DCs) and DC2 DCs. However, the viral DNA is not sufficient for DC immune activation. The viral capsid protein in association with viral DNA, controls the cGAS-mediated innate immune response in DCs through an unresolved mechanism. While the incoming HIV-2 capsid is permissive for recognition of the viral DNA before viral integration, the HIV-1 capsid limits viral DNA recognition before integration. We hypothesized that a capsid-binding factor with preferential binding to the HIV-2 capsid could be implicated in viral recognition. In a screen, we identified a previously unreported capsid-binding protein (CBP). CBP binds directly to HIV capsid, with more affinity for HIV-2 than HIV-1 capsid. Using multiple depletion approaches, we find that CBP is neither a restriction factor nor a facilitating factor of viral replication. Instead, through multiple approaches, we find that CBP is an essential innate immune sensor of the HIV capsid in the nucleus of DCs. Association of viral capsid recognition with viral DNA sensing in the nucleus constitutes an innate strategy to achieve specific distinction of viruses from self DNA in the nucleus.

240. Treatment Options for Human RSV: Small Molecules, Antibodies or Something in Between?

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Infections with human respiratory syncytial virus (RSV) result in a significant clinical burden worldwide, most notably in children and the elderly. When RSV infects the lower respiratory tract, patients often develop pneumonia and bronchiolitis and may require hospitalization. Without an approved RSV-specific antiviral drug available, except for the prophylactic use of a monoclonal antibody that targets the fusion protein, the clinical care or RSV patients is limited to supportive care and, unfortunately, not always successful. However, promising new small molecule antivirals that target the RSV fusion protein or polymerase are now being evaluated in the clinic. In addition, a battery of human monoclonal antibodies have been developed that target the fusion and glycoprotein of RSV. Although primarily intended for prophylactic use, some of these monoclonal antibodies have a therapeutic effect in animal models of RSV. The presentation will highlight some of the most recent candidate small molecule anti-RSV drugs as well as the tremendous progress that has been made in the area of antibody-based RSV therapeutics, including single domain antibodies.

241. Strategies for an HIV Cure: Early Lessons from Shock and Kill Trials

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Pharmacologically induced expression of latent virus is investigated as part of a cure for HIV-1 infection. Recent data from clinical trials show that short-term administration of a latency-reversing agent (LRA) may increase HIV-1 transcription, HIV-1 protein expression, and plasma HIV-1 RNA. So far, reversal of HIV-1 latency by histone deacetylase inhibitors and other LRAs has not been associated with a reduction in the size of the latent reservoir. Possible explanations for the lack of an effect on the size of the latent HIV-1 reservoir include insufficient immune response against virus-expressing cells, the presence of cytotoxic T lymphocyte (CTL) escape variants, and/or an insufficient degree of latency reversal achieved with current approaches. Importantly, these early studies of LRAs were primarily designed to investigate their ability to perturb the state of HIV-1 latency. Newer studies have attempted to either combine LRAs with interventions such as therapeutic HIV-1 vaccines aimed at improving the killing of reactivated cells or to test compounds such as TLR7 and TLR9 agonists which may have dual effects as both LRAs and enhancers of antiviral immunity. This talk will focus on the lessons we have learned from these early « shock and kill » trials and review recent published as well as unpublished data.

242. ANTIVIRALS, a European Training Network to Train the Next Generation of Experts in Antiviral Drug Development

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Antiviral drug development requires a detailed understanding of the molecular mechanisms of virus replication in conjunction with a multidisciplinary approach that is based on expertise and technology from the fields of molecular virology, biochemistry, structural biology, computer-aided drug design, and medicinal chemistry. Few, if any, European universities or research institutes have the broad know-how required to deliver a comprehensive and intersectoral training programme that covers the full spectrum of disciplines important for antiviral drug development. To fill this gap, 7 European research institutes - including academic partners with complementary expertise in molecular virology as well as academic partners specialised in developing novel antiviral compounds and strategies (Utrecht University, Leiden University Medical Center, University of Heidelberg, University of Leuven, Cardiff University, Marseille University and University of Vienna) - and 4 industrial partners (AiCuris, a pharmaceutical R&D company specialised in antiviral drug discovery, Prestwick Chemical, a company specialised in medicinal chemistry, and 2 SMEs, Complix and Virovet) have established the "ANTIVIRALS" consortium, a EU-funded Marie Curie European Training Network (www.antivirals-etn.eu).

I will discuss how we provided a multi-national group of 15 Early Stage Researchers (ESRs) with state-of-the-art scientific knowledge and technological capabilities in the disciplines mentioned above as well as generic skills (e.g. team skills, science communication, dissemination & societal outreach, innovation & entrepreneurship, IPR & innovation) so that ESRs are able to understand and speak the different languages of academia and industry. These capabilities are further being developed in the ESRs' Individual Research Projects, which focus on different viruses such as Dengue virus, Zika virus, chikungunya virus, MERS-coronavirus, RSV, enteroviruses, and HBV (results of these projects will be presented by each of these ESRs by oral or poster presentation during this ICAR conference).

243. Influenza Virus Infection, Neuraminidase Inhibitor Treatment, and Emergence of Drug Resistance in an Immunocompromised Ferret Model

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Influenza viruses can cause severe life-threatening infections in high-risk patients, including young children, the elderly and patients with compromised immunity due to underlying medical conditions or immunosuppressive treatment. The impaired immunity of these patients causes prolonged virus infection and combined with antiviral treatment facilitates the emergence of viruses with resistance mutations. The diverse nature of the immune status of the patient population makes them a challenging group to study the impact of influenza virus infection and the efficacy of antiviral therapy. Immunocompromised ferrets may represent a suitable animal model to assess influenza virus infection and antiviral treatment strategies in immunocompromised hosts. Ferrets were given a daily oral solution of mycophenolate mofetil, tacrolimus and prednisolone sodium phosphate to suppress their immune system. Groups of immunocompromised and immunocompetent ferrets were inoculated with influenza virus and subsequently treated with Oseltamivir or left untreated. Virus replication during the course of infection was measured. All immunocompromised ferrets had prolonged presence of viral RNA and a higher total amount of virus shedding compared to immunocompetent ferrets. Although Oseltamivir reduced virus shedding, it also resulted in the emergence of neuraminidase resistance substitutions in the animals. No compensatory mutations that could be associated with the emergence of resistance mutation were detected. The immunocompromised ferret is a promising model to study new antiviral strategies against A/H1N1 and A/H3N2 virus infection and the emergence of antiviral resistance in immunocompromised hosts.

244. Epidemic Arboviral Diseases: Implications for Research and Public Health Infrastructure

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For decades, arbovirus diseases were considered only minor contributors to global mortality and disability. As a result, low priority was given to arbovirus research investment and related public health infrastructure. The last five decades, however, have witnessed an unprecedented emergence of epidemic arbovirus diseases, notably dengue, chikungunya, yellow fever and Zika, by exploiting the triad of the modern world: urbanization, globalization and international mobility. The public health emergency of Zika, and the threat of global spread of yellow fever, combined with the resurgence of dengue and chikungunya, constitute a wake-up call for governments, academia, funders and the World Health Organization to strengthen programmes and enhance research in Aedes-transmitted diseases. The common features of these diseases should stimulate similar research themes for diagnostics, vaccines, biological targets and immune responses, environmental determinants and vector control measures. Combining interventions known to be effective against multiple arboviral diseases will offer the most cost-effective and sustainable strategy for disease reduction. New global alliances are needed to allow combining such efforts and resources for more effective and timely solutions.

245. Hepatitis C virus: Problem Solved. And Now?

Jean-Michel Pawlotsky[1]
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Now that effective cures for HCV are available, what now?

246. Monitoring, Predicting and Altering the Evolution of RNA Virus Populations

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RNA viruses generate huge mutant swarms that allow rapid evolution within a host. While NGS technologies allow us to identify the thousands of mutants in a virus population, identifying which mutations and composition of variants is relevant to infection remains a challenge. We combined mathematical dimension reduction methods and new mathematical matrix algorithms to identify biological signals in NGS data to monitor RNA virus evolution. We show that despite the high theoretical dimensionality of sequence space, the biologically relevant sequence space is of low dimensionality and can track virus evolution. We reconstruct genotype-phenotype landscapes and show that minority variants contribute significantly to fitness, and allows for better prediction of phenotype. We illustrate how distribution-based modeling of sequence space time dynamics can help predict virus evolution and alter it in antiviral approaches.

247. Passive Antibody Therapies against Endemic, Emerging and Re-Emerging Viral Infectious Diseases

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Monoclonal antibodies have revolutionized the treatment of several human diseases, including cancer, autoimmunity and inflammatory conditions and represent a new frontier for the treatment of infectious diseases. In the last decade, new methods have allowed the efficient interrogation of the human antibody repertoire from immune individuals and the swift isolation of potent neutralizing monoclonal antibodies, including rare ones able to target conserved sites in highly variable viruses. In addition, new antibody engineering technologies have been and are developed to improve half-life and effector functions, to generate multi-specific antibodies, thus providing a potential for further augmented in vivo activity and reduced risk of viral escape. In the coming years neutralizing monoclonal antibodies have therefore the potential to be developed for the prophylaxis and therapy of viral infections, including those caused by newly emerging pathogens with a pandemic potential. In this presentation, Dr. Corti will provide an overview of the specificity, antiviral and immunological mechanisms of action of neutralizing monoclonal antibodies directed against influenza A virus, RSV, rabies virus and Zika virus. In addition, the presentation will also introduce the concept of antibody-driven vaccine design, i.e. how the analysis of the human immune response has provided an innovative approach to the identification of protective antigens which are the basis for the design of vaccines capable of eliciting effective B-cell immunity.

248. Mechanisms of Immune Dysfunction in Chronic Hepatitis B and Possible Immune Therapies

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Chronic hepatitis B develops as the consequence of viral persistence mechanisms and unique mechanisms of local regulation of immune responses in the liver that together are responsible for failure of anti-viral immunity to eliminate HBV infection. While the liver harbors unique cell populations with immune functions, both liver-resident as well as bone-marrow derived, the outcome of immune responses locally in the liver often is immune tolerance rather than immunity. This talk will discuss our present knowledge on immune regulatory mechanisms in the liver in general and more specifically the mechanisms known to perturb HBV-specific immunity. Moreover, it will be addressed how local T cell immunity in the liver can be specifically increased and which role monocyte-derived cell populations have in achieving successful immune surveillance in the liver.

250. USC-087, an HPMPA Prodrug, Prevents Varicella Zoster Virus Replication in Human Skin in Culture and in a Mouse Model

Jennifer Moffat, Ph.D.[1], Dongmei Liu, Ph.D.[1], Mark Prichard, Ph.D.[2], Jiajun Fan, Ph.D.[3], Jinglei Lyu, Ph.D.[3], Boris A. Kashemirov, Ph.D.[3], and Charles E. McKenna, Ph.D.[3]
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USC-087 is an *N*-C_{16} alkyl tyrosinamide phosphonate ester prodrug modification of HPMPA. It was previously reported to have good bioavailability, a long half-life in rodents, and broad spectrum antiviral activity against DNA viruses (poxvirus, adenovirus, herpesvirus). USC-087 is highly potent against varicella zoster virus in cultured cells (EC_{50} 0.005 μM, CC_{50} 0.31 μM), and so we investigated its efficacy in full-thickness skin organ culture (SOC) and in SCID-Hu mice. USC-087 was effective in SOC (EC_{50} approx. 2.9 μM, 2.0 μg/mL). *In vivo* assays were performed using doses 8.2 and 24.7 mg/kg, given once daily, via oral or subcutaneous routes, and starting at the time of virus infection. USC-087 prevented VZV spread *in vivo* (ANOVA, $p \leq 0.004$), and the effects were dose-dependent. The subcutaneous route was more effective than the oral route at equivalent dose concentrations, and at 8.2 mg/kg gave a comparable reduction in VZV yield to 10 mg/kg cidofovir. USC-087 was well-tolerated, although the highest subcutaneous dose caused weight loss (approx. 20%). The effects of treatment schedule and vehicle for oral dosing will be discussed. Phosphonate antiviral prodrugs bypass the initial phosphorylation step mediated by viral thymidine kinase, making them useful against strains that acquire resistance mutations in the TK gene. Thus, the effectiveness of USC-087 against an isogenic TK-mutant VZV strain is of interest. In conclusion, USC-087 administered s.c. potently inhibits VZV spread *in vivo* in the SCID-Hu mouse model. This work was supported in part by Public Health Service Contracts HHSN272201100016I, HHSN272201000023I, and HHSN272201700030I, NIAID, NIH.

252. A Novel Flavivirus Inhibitor, DVI-1, Discovered Through High-Throughput Screening (HTS) with Dengue Reporter Viruses

Andrew Yueh, Ph.D.[1]
[1]**National Health Research Institutes, Taiwan**

Dengue virus (DENV) is a global health problem, affecting approximately 3.9 billion people in the world. Therefore, the development of therapeutic agents to treat this epidemic disease is urgently needed since some risk concerns have been raised for the only licensed dengue vaccine Dengvaxia. In this report, we identified a potential small-molecule inhibitor, DVI-1, via cell-based high-throughput screening of 12,000 compounds using DENV-2 reporter viruses. DVI-1 reduced the dengue virus yields in virus-infected cells, showing a 50% effective concentration (EC_{50}) of 0.48 ± 0.06 μM. Without detectable cytotoxicity, the compound not only inhibited all four serotypes of DENV but also Japanese encephalitis virus. Time-addition experiments further indicated that DVI-1 inhibits viral entry stages (-24 to +2 hr) rather than at the viral translation stages (+6 hr). Sequencing analyses of several individual clones derived from DVI-1-resistant reporter viruses, which revealed a consensus amino acid substitution in the stem region of the E protein. Introduction of the DVI-1-resistant mutation (within E gene) into the DENV reporter viruses conferred over 14.8-fold EC_{90} shift to DVI-1. Importantly, the combination of DVI-1 with ribavirin, a viral replication inhibitor, showed synergistic reduction of DENV-2 in virus-infected cells. Our results identify an effective small-molecule envelope inhibitor, DVI-1, which may be developed to anti-DENV drug candidate in the future.

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