ICAR 2022

PROGRAM & ABSTRACTS

HYBRID CONFERENCE

Seattle, WA, USA
RENAISSANCE SEATTLE HOTEL

ISAR

MARCH 21 – 25
2022

35th INTERNATIONAL CONFERENCE ON ANTIVIRAL RESEARCH (ICAR)

Hosted by the **International Society for Antiviral Research (ISAR)**

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ICAR2022

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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 International Conference on Antiviral Research (ICAR). The Society, now in its 35th year of existence, has members representing 30 countries. To become an ISAR member, visit our website at **www.isar-icar.com**.

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Confirmed sponsors as of March 11, 2022

ICAR2022

PREMIER PLATINUM

Bill & Melinda Gates foundation

Gilead* Creating Possible

pad pandemic antiviral discovery

SAPPHIRE

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Additional support provided by:

Journal of General Virology

Sage Publishing
(for Young Investigator Poster Award)

*Supported by Gilead who provided funding. Gilead has had no input into the content of the materials used at this meeting/conference.

Virtual Platform Information

ICAR2022

ICAR2022 – a hybrid conference – will offer both in-person and virtual programming options. Please find below details to assist you with navigating the ICAR2022 virtual platform.

How do I access the virtual platform?

All registered attendees (onsite and virtual) will receive log-in details on Monday, March 21. The ICAR2022 virtual platform will work best with the latest version of Google Chrome. Please ensure you use Google Chrome to access the platform to minimize technical problems.

ICAR 2022 Virtual Platform Link:
https://portalapp.gravesshow.eventsair.com/VirtualAttendeePortal/icar-2022/onair/login

When will conference content be available?

Content will be available to all registered attendees starting Monday, March 21, 2022.

How long will conference content be available on the virtual platform?

All registered attendees will be able to access on-demand content through April 25, 2022.

How do I watch and participate in the live sessions?

Starting Monday, March 21 you may log into the virtual platform, where you will see the timeline of all scheduled live sessions. You can **"Preview"** a session at any time prior to its start time from the timeline. Once the session is live, the **"Preview"** button will change to **"Join"**. When you join a session, you will be able to see the main screen, the speaker(s), and their presentation as well as the Live Q&A chat box where you may ask real-time questions.

The detailed schedule can be found in this Program Book, the conference website, or the virtual platform. All scheduled times and dates for ICAR2022 live programming are listed in Pacific Time.

Will the live sessions be recorded?

If you are unable to attend the live programming, recordings will be posted for on-demand viewing within 24-48 hours following the live session. Please note that some special sessions may not be recorded.

How do I view the posters?

All posters (even those being presented onsite) will be available as electronic posters for on-demand viewing by attendees as their own schedule permits. Q&A for virtual posters will be available through the virtual platform chat board associated with each poster. There will be no live Q&A for virtual posters.

How do I interact with poster presenters if I have questions?

Each poster presenter will have a chat message board on their poster page that will be monitored by the presenting author. Presenters are expected to check their chat board at least twice daily during the conference week. We encourage attendees to leave feedback, questions, ask to be contacted for more information, or even leave a simple 'hello' to check in, so that they know you visited. There will be no live Q&A for virtual posters.

Who do I contact if I have problems?

For technical questions about accessing or using the platform:

- While in the platform, click the "Live Support" button on the top menu bar
- Contact Production Team at info@gravesshow.com

For questions about registration or other Conference items, please contact ICAR Staff at info@isaricar.com.

Are there training videos?

Please visit our website and click on the Frequently Asked Questions page. This page will be updated frequently with details and tips for ICAR2022 to include various training videos to help you become familiar with the virtual platform.

Speed Networking

OPEN TO ALL ATTENDEES *(Via the Virtual Platform)*

Monday, March 21 • 11:15 – 11:45 AM PT

Ready to mix things up? Join us for this 30-minute session where you will be randomly matched with a small group of attendees for FIVE minutes for a video chat. When time is up, you will be randomly matched with a new group of individuals, giving you the chance to meet people you have never met before, and to make some new lasting connections! Do not miss this interesting opportunity to mix things up in your social world.

Women in Science Roundtable Discussion

IN-PERSON EVENT ONLY

Monday, March 21 • 12:00 – 1:45 PM PT

MADISON SALON A (2nd Floor)

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 an exciting exchange of ideas with a panel of expert antiviral research scientists as well as other event participants.

Registration will occur from noon-12:30 PM. It is open to both women and men and will feature discussions on issues facing women in antiviral research. Drinks and light food will be provided.

Opening Session and Plenary Speakers

Monday, March 21 • 2:00 – 4:15 PM PT

COURTYARD BALLROOM

Ken Duncan, *Bill & Melinda Gates Foundation*

What Role Can Foundations Play in Driving Innovation Globally in Antiviral Development?

Carl W. Dieffenbach, *National Institute of Allergy and Infectious Diseases, National Institutes of Health*

Drug Development for Viruses of Pandemic Potential, Where Are We Now and Where Are We Going?

Opening Reception

IN-PERSON EVENT ONLY

Monday, March 21 • 5:30 – 6:30 PM PT

MADISON BALLROOM (2nd Floor)

Direclty following the opening session, all onsite attendees are invited to join us at the Opening Reception where we can finally mix and mingle with each other in person!

PeckaKucha Competition

Tuesday, March 22 • 11:30 AM – 12:30 PM PT
COURTYARD BALLROOM

Get ready to be entertained and informed as finalists present their PechaKucha presentations. Not familiar with PechaKucha? The presenter has 15 slides, each on the screen for only 20 seconds. The slides advance automatically and the presenter has to keep up with the slides, as they won't have control. Be prepared for some humor, a few surprises and maybe something unexpected. Prizes will be awarded by a panel of judges to the top three finalists.

Late-breaking Oral Presentations

Wednesday, March 23 • 12:15 – 1:00 PM PT
COURTYARD BALLROOM

New this year! This session will feature high quality presentations containing the most recent data with cutting edge implications and impact.

Closing Dinner Event
IN-PERSON EVENT ONLY

Thursday, March 24 • 7:00 – 10:00 PM PT
COLUMBIA TOWER CLUB (less than 2 blocks from hotel)

Join us to network with your colleagues while taking in spectacular views of the Seattle skyline and Puget Sound from the 76th floor of Seattle's tallest building. Winners of the Poster Awards and PechaKucha Competition will also be announced. Please note that you must have submitted your RSVP to attend this event.

Career Development Interactive Workshop

Friday, March 25 • 8:30 – 9:15 AM PT
COURTYARD BALLROOM

THE POWER OF MENTORING

Prof. Harmit Malik, *Fred Hutchinson Cancer Research Center, Seattle, WA*

During this career development session, Prof. Harmit Malik will talk about his career path, the importance of several mentors for his career and how he developed his own mentoring style. Onsite and virtual attendees will have the opportunity to ask questions about mentoring and career development to Prof. Malik. At the end of the session, onsite attendees will have the opportunity to informally network with the speaker and with the other attendees to make new contacts and ask follow-up questions. The career development session will be available to both onsite and virtual attendees.

NEW THIS YEAR!

ICAR2022

We are crowdsourcing our Conference photos, so all onsite attendees are encouraged to share their best photos from the Conference (sessions and networking events) using **#liveICAR2022**.

PLEASE DO NOT INCLUDE ANY PRESENTATION CONTENT IN THE BACKGROUND OF YOUR PHOTOS.

Be Sociable – Share!

#ISAR #ICAR2022 #AntiviralResearch

@ISARICAR

Like us at www.facebook.com/ISARICAR

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As a virtual attendee, you may log into the virtual platform, and join the live sessions starting Monday, March 21. All posters (even those being presented onsite) will be available as electronic posters for on-demand viewing by attendees as their own schedule permits. Refer to the Virtual Information page for additional details.

Monday, March 21, 2022

TIME *(Pacific Time)*	EVENT	LOCATION
12:00 PM – 1:45 PM	**Special Event: Women in Science Roundtable** (IN-PERSON EVENT ONLY)	MADISON BALLROOM (2nd floor)
2:00 PM – 4:15 PM	**Opening Session and Plenary Speakers**	COURTYARD BALLROOM
4:15 PM – 4:30 PM	**Break**	COURTYARD FOYER
4:30 PM – 5:30 PM	**Gertrude Elion Memorial Award Lecture**	COURTYARD BALLROOM
5:30 PM – 6:30 PM	**Opening Reception** (IN-PERSON EVENT ONLY)	MADISON BALLROOM (2nd floor)

Tuesday, March 22, 2022

TIME *(Pacific Time)*	EVENT	LOCATION
8:30 AM – 9:15 AM	**Antonín Holý Memorial Award Lecture**	COURTYARD BALLROOM
9:15 AM – 11:00 AM	**Non-coronavirus Respiratory Viruses**	COURTYARD BALLROOM
10:15 AM – 10:30 AM	**Break**	COURTYARD FOYER
11:00 AM – 12:00 PM	**PechaKucha Competition**	COURTYARD BALLROOM
1:30 PM – 2:15 PM	**William Prusoff Memorial Award Lecture**	COURTYARD BALLROOM
2:15 PM – 5:00 PM	**Retroviruses and Other Viruses**	COURTYARD BALLROOM
3:45 PM – 4:00 PM	**Break**	COURTYARD FOYER
5:00 PM – 7:00 PM	**Poster Session 1** *Light refreshments provided* (IN-PERSON SESSION ONLY)	COMPASS ROOMS (3rd floor)

Wednesday, March 23, 2022

TIME *(Pacific Time)*	EVENT	LOCATION
8:30 AM – 9:15 AM	Antonín Holý Memorial Award Lecture (2020)	COURTYARD BALLROOM
9:15 AM – 12:15 PM	Broad Spectrum Antiviral Drugs and Pandemic Preparedness	COURTYARD BALLROOM
10:15 AM – 10:30 AM	Break	COURTYARD FOYER
12:15 PM – 1:00 PM	Late-breaking Oral Presentations	COURTYARD BALLROOM
1:00 PM – 3:00 PM	Poster Session 2 *Lunch provided* (IN-PERSON SESSION ONLY)	COMPASS ROOMS (3rd floor)

Thursday, March 24, 2022

TIME *(Pacific Time)*	EVENT	LOCATION
8:30 AM – 9:15 AM	Women in Science Award Lecture	COURTYARD BALLROOM
9:15 AM – 12:00 PM	Arboviruses	COURTYARD BALLROOM
10:15 AM – 10:30 AM	Break	COURTYARD FOYER
12:00 PM – 12:15 PM	ISAR Annual Business Meeting	COURTYARD BALLROOM
2:00 PM – 2:45 PM	Diversity Speaker Award Lecture	COURTYARD BALLROOM
2:45 PM – 5:45 PM	Coronaviruses	COURTYARD BALLROOM
3:45 PM – 4:00 PM	Break	COURTYARD FOYER
7:00 PM – 10:00 PM	Closing Dinner Event (IN-PERSON SESSION ONLY)	COLUMBIA TOWER CLUB 701 5th Avenue (2 blocks from hotel)

Friday, March 25, 2022

TIME *(Pacific Time)*	EVENT	LOCATION
8:30 AM – 9:30 AM	Special Event: Career Development Interactive Workshop	COURTYARD BALLROOM
9:30 AM – 12:00 PM	Hepatitis and Herpes Viruses	COURTYARD BALLROOM
10:30 AM – 10:45 AM	Break	COURTYARD FOYER
12:00 PM – 12:30 PM	Shotgun Presentations and Closing Session	COURTYARD BALLROOM

GERTRUDE ELION MEMORIAL AWARDEE

George Painter

Developing an Oral Antiviral Agent in a Pandemic: The Evolution of Molnupiravir (EIDD-2801, MK-4482) as a Treatment for COVID-19

Dr. George Painter holds the positions of Professor of Pharmacology and Chemical Biology, **Emory University School of Medicine**, CEO of DRIVE (**Drug Innovation Ventures at Emory**) and Executive Director of The Emory Institute for Drug Development. In these roles he has led the establishment of a freestanding drug discovery and development company inside the university structure, which currently has drugs under development for COVID-19 and enterovirus infections. Over the past 30 years he has played a major role in the discovery, development and implementation of modern antiviral therapy. Prior to coming to Emory, he was a cofounder of the biotechnology company Chimerix, Inc. (NASDAQ:CMRX) and served as its President and Chief Executive Officer for nine years. During his tenure, the company developed a drug for the prophylaxis and treatment of smallpox infection, Tembexa, which was approved by the FDA in June of this year. Before coming to Chimerix, he was a founding member of the management team of Triangle, Inc., an HIV company where he led the development of the key HIV drug emtricitabine. Prior to entering the biotechnology sector, Dr. Painter held senior management positions in two global pharmaceutical companies, Burroughs Wellcome Co. and GlaxoWellcome, where he led the discovery, development and commercialization of antiviral agents to treat HIV and HBV. He is an inventor on over one hundred fifty patents, many of which have led to approved commercially-available antiviral drugs or combinations of antiviral drugs for the treatment of HIV, hepatitis B, smallpox, and coronavirus infections. Dr. Painter has a PhD in Organic Chemistry and a Master's degree in Physical Organic Chemistry from Emory University. He was a post-doctoral fellow at the California Institute of Technology.

ANTONÍN HOLÝ MEMORIAL AWARDEE

Mark von Itzstein

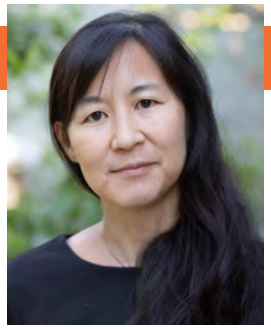
Structure-guided Antiviral Drug Discovery – A Tale of Two Viruses

Prof. Mark von Itzstein AO has international standing in glycoscience, medicinal chemistry and drug discovery, particularly in the area of anti-infective drug discovery. He has established an internationally-recognised research program that is investigating the discovery of novel anti-microbial drugs, including novel anti-influenza drugs, anti-parainfluenza and anti-cancer drugs based on carbohydrate-related pathways. In 2020 he was awarded the prestigious National Health and Medical Research Council Investigator L3 award.

He established and heads the **Institute for Glycomics** at **Griffith University** on the University's Gold Coast Campus. The Institute is the only multidisciplinary translational glycoscience research centre in Australia and one of a handful in the world. Prof. von Itzstein has now attracted over $50 million in the establishment of the Institute.

Prof. von Itzstein's research led to the discovery of the anti-influenza drug, Relenza®. This drug was designed, synthesised and biologically evaluated (in vitro) in Prof. von Itzstein's laboratory. This discovery is considered to be the most significant outcome and flagship in glycotherapeutic drug development in the last century and has consolidated the world platform of using carbohydrates and carbohydrate-recognising proteins as drugs and drug discovery targets, respectively. Relenza® is the first 'designer' anti-viral drug in the world.

Prof. von Itzstein's contributions to the carbohydrate sciences and medicinal chemistry have been internationally recognised by continued conference invitations and requests to write significant chapters on carbohydrate science and drug discovery and act as a book editor/co-editor. Prof. von Itzstein has published over 300 contributions and is one of Australia's leading medicinal chemists.



WILLIAM PRUSOFF MEMORIAL AWARDEE

Priscilla Yang

Targeted Protein Degradation as an Antiviral Strategy

Priscilla Yang earned her PhD in Bio-organic Chemistry at the University of California, Berkeley. Following postdoctoral training in viral immunology at Scripps Research, she started her independent career at Harvard Medical School, where her laboratory combined chemical and pharmacological approaches to address fundamental and translational problems in virology. She is currently Professor in the Department of Microbiology and Immunology at the **Stanford University School of Medicine** where she focuses on leading and mentoring a multidisciplinary group of scientists focused on discovery and validation of new antiviral targets; identifying new strategies to achieve broad-spectrum activity and to avoid antiviral resistance; and investigating the function of lipid membranes in RNA virus replication. She is a strong advocate for diversity, equity, and inclusion in science and is proud to have been the recipient of the inaugural ISAR Women in Science Award.

WOMEN IN SCIENCE AWARDEE

Christina Spiropoulou

Fighting Viral Hemorrhagic Fevers: From the Benches to the Trenches

Christina Spiropoulou is a virologist with an extensive background in basic and translational research applied to the development of medical countermeasures for hemorrhagic fever viruses. She currently serves as the Deputy Chief of the Viral Special Pathogens Branch at the **US Centers for Disease Control and Prevention** in Atlanta and lead scientist for the Molecular Pathogenesis and Therapeutics Team. For the past 29 years, her research interests have focused on hemorrhagic fever viruses, a diverse group of zoonotic RNA viruses that includes Ebola, Lassa, Nipah, Crimean-Congo hemorrhagic fever, Rift Valley fever, and tick-borne encephalitis viruses. During her tenure at CDC, she participated in the discovery of the pathogenic New World hantaviruses and has deployed to several VHF outbreaks. Her team's current projects focus on scientific questions with the potential to lead to development of prototype vaccines and identification of targets for antivirals or immunotherapeutics.

DIVERSITY AWARDEE

J. Victor Garcia-Martinez

The COVID-19 Pandemic: A View from the Bench

Dr. J. Victor Garcia-Martinez, **University of North Carolina, Chapel Hill**, uses humanized mouse models, including recently developed humanized-lung mice, to study the pathogenesis of human-specific viruses and therapeutic interventions. Dr. Garcia is using these models to study an array of human respiratory pathogens including RSV, CMV, influenza, MERS, SARS, SARS-CoV-2 and tuberculosis. He has trained over 13 PhD students 40 postdoctoral fellows (past and present). His trainees hold positions in industry, government and academic institutions. Dr. Garcia is a Fellow of the American Academy of Microbiology and his contributions to Inclusion and Diversity have been recognized by the American Academy of Microbiology and the American Society for Cell Biology. Dr. Garcia is also the Director of the International Center for the Advancement of Translational Science.

Throughout evolution, humans have been challenged by viral pathogens new to the species. The immune system mounts an adequate response that protects us from the fatal consequences of infection. However, in some instances viruses can circumvent the immune system and cause fatal diseases such as cancer (EBV, KSHV, HCV), hemorrhagic fever (Ebola virus), AIDS (HIV) and pandemics (Influenza, SARS, MERS, SARS-CoV-2). Understanding the host pathogen relationship at a cellular level provides rational approaches to therapy and vaccine development. It also provides a better understanding of human biology and immunology. Dr. Garcia is interested in how human viruses cause human cancer, how HIV causes AIDS and why the immune system is not able to control viral infections and how coronaviruses cause disease in humans. His working hypothesis is that specific viral genes are the key determinants of viral pathogenesis and are responsible for disease progression. To evaluate the role of these genes in disease progression, he has developed in vivo models that recapitulate specific aspects of viral infection. His emphasis has been placed on precision animal models where human specific pathogens can be studied and where novel therapeutic interventions can be evaluated.

The 2022 Chu Family Foundation Scholarship Awardees

ICAR2022

ISAR and The Chu Family Foundation (TCFF) Committee are pleased to announce the winners of the 2022 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.

2022 TCFF AWARDEES

Laura Taracón Díez

MADRID, SPAIN

Having graduated in Biochemistry at the University of Navarra (Spain) in 2013, Laura Tarancón-Díez has been interested in the field of HIV infection since a training in the Department of Human Protein Sciences, University of Geneva (Switzerland), working on the study of anti-HIV analogues.

After a Master's degree in Biotechnology and Molecular Genetics, she completed her PhD in 2019 at the University of Seville (Spain) under the supervision of Manuel Leal and Ezequiel Ruiz-Mateos on the study of immunological parameters and potential mechanisms associated with the spontaneous control in the setting of the elite controller patient. In 2019 she joined as postdoc the Laboratory of Molecular ImmunoBiology at Hospital Gregorio Marañón in Madrid (Spain) directed by Mª Ángeles Muños-Fernández.

Her work is now focused on the study of immunovirological dynamics associated to early antiretroviral treatment response in vertically HIV-1 infected children and vaccine responses in immunosuppressed children including HIV-1-children. Additionally, since COVID-19 pandemic started, she is also analysing the immune and vaccine responses against SARS-CoV2 infection based on multiple risk factors such as pregnancy, age, and HIV co-infection.

The funding provided by the Chu Family Scholarship will allow her to attend, in addition to ICAR2022 in Seattle, the 2022 edition of the Conference on Retroviruses and Opportunistic Infections (CROI) dedicated also to SARS-CoV2 infection where she will share her recent study about the inflammatory consequences of SARS-CoV2 infection during pregnancy on women and exposed newborns.

Ana Lucia Rosales Rosas

LEUVEN, BELGIUM

Ana Lucia Rosales completed her undergraduate studies in Biology at the KU Leuven (Belgium), after which she became a PhD student in Prof. Leen Delang's group, the Mosquito Virology Team, at the Rega Institute for Medical Research (KU Leuven, Belgium). Ana's research focus is on arboviruses, specifically on the interplay between antiviral drugs and the mosquito vector. She is also interested in developing new tools to study arbovirus infection. For that purpose, the funding provided by the Chu Family Foundation will allow her to attend the "Applications of Organoid Technology" course at MDI Biological Laboratory (Bar Harbor, ME). Skills and experience gained during this course will fuel the start of a fascinating and exploratory project at the Mosquito Virology Team: a mosquito gut organoid system.

Ashleigh Shannon

MARSEILLE, FRANCE

Ashleigh Shannon completed her PhD in July 2017 in the lab of Prof. Paul Young at the University of Queensland, Australia. Her thesis research was focused on the structure and activity of the dengue virus NS3 protease, and its potential as a drug target. Upon completion, Ashleigh spent a year in a research and development role at VAXXAS (Translational Research Institute, Queensland), a biotech company which uses a needle-free platform, known as the Nanopatch, for vaccine delivery. In 2018, she was awarded an Australian Endeavour Research Fellowship, which allowed her to move to the lab of Dr Bruno Canard, at the AFMB, Aix-Marseille Université, France. She is now in her third year as a post-doctoral researcher in this lab, focused on the structure and function of enzymes involved in viral replication and RNA capping, and their use in antiviral drug design. Her current research is specifically focused on the polymerase and nucleotidyl-transferase enzymes of the Coronaviridae family.

Ashleigh will use the funds from the Chu Family foundation scholarship to attend the ICAR meeting, in addition to the Plus Strand RNA virus Keystone symposia. The remaining money will be used to attend a training program for NGS sequencing analysis. This scholarship will help her in her goal to obtain a permanent position with the CNRS (Centre national de la recherche scientifique) at the end of the year.

Ralf Altmeyer

Inducing Phase Transition in Viral Condensates. A Novel Antiviral Strategy

Dr. Ralf Altmeyer, CEO of **Medusa Therapeutics Limited**, graduated from University of Tubingen in Germany and did his PhD and postdoctoral training at Institut Pasteur Paris. His scientific career focuses on pathogen-host interactions and development of innovative drugs. He directed the Hong Kong University – Pasteur Research Centre (2003-2006), CombinatoRx-Singapore (2006-2009), Institut Pasteur Shanghai – Chinese Academy of Sciences (2010-2014) and the Helmholtz International Lab at Shandong University (2015-2019). Using approved drug repurposing his team identified suramin as an entry inhibitor of EV-A71 (Ren, 2014, EMI; Ren, 2017 SciRep), the causative agent of Hand Foot Mouth Disease in children. Suramin is currently in clinical development in China. More recently his team worked on RSV, the leading cause of pneumonia in children. His team identified the transcription factor M2-1 as a target for therapeutic intervention (Bailly, 2016, SciRep) leading to the recent discovery that RSV inclusion bodies are LLPS biomolecular condensates which can be hardened by small molecules resulting in reduction of virus in the lungs of infected animals (Risso-Ballester, 2021, Nature). This study provided proof of concept that viral condensates can be targeted by drug-like small molecules for the development of fast-acting therapeutics against acute viral infections.

Please note the following speaker, Lee D. Arnold, is unable to attend. This presentation will now be given by Uri Lopatin.

Lee D. Arnold

Profile of PBI-0451 an Orally Administered 3CL Protease Inhibitor of SARS-CoV-2 for COVID-19

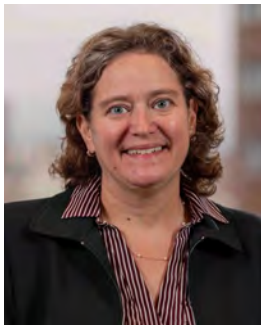
Lee D. Arnold, PhD, CSO, **Pardes Biosciences**, has held scientific leadership roles in diverse public and private companies. Most recently, Lee was the Senior Vice President of Research at Kinnate Biopharma. Prior to Kinnate, Lee was CSO at Assembly Biosciences, where he was a co-inventor of vebicorvir and ABI-H2158 for hepatitis B. As CSO of Coferon, Lee pioneered novel bivalent therapeutics based on the in vivo self-assembly of ligands upon their targets. Earlier he was Vice President of US Research at OSI Pharmaceuticals (now part of Astellas), Project Team Leader at BASF/Abbott Bioresearch Center, and held positions at Pfizer and Syntex.

Altogether, Lee has played an integral role in delivering 14 IND-track drug candidates into development in oncology and virology, including numerous first-in class drugs including TARCEVA (erlotinib), OSI-906 (linsitinib; IGF1R inhibitor), and OSI-027 (TORC1/TORC2 inhibitor). Lee has over 85 published patent applications, and is co-author on 38 peer-reviewed papers. He received an honors BS in chemistry at the University of Waterloo, and a PhD in organic chemistry from the University of Alberta.

Christian Callebaut

Lenacapavir: The First Clinically Active Long-Acting Inhibitor of HIV Capsid

Dr. Christian Callebaut is a Sr. Director in the Virology Department at **Gilead** and the lead for HIV Clinical Virology. He earned his PhD in Virology from the University of Paris, did his doctoral training in the AIDS & Retrovirus Department at the Institut Pasteur Paris, and was a post-doctoral fellow at Gladstone Institutes of Virology and Immunology. During his tenure at Gilead, Christian has played an integral role the discovery of several HIV drugs, and contributed to the development, regulatory filing and post-approval support of several antiretrovirals, such as Truvada®, Stribild®,Vitekta®, Tybost®, Genvoya®, Odefsey®, Descovy®, Biktarvy®. More recently, he has been the lead Virologist for the development of lencapavir (LEN, GS-6207), a novel HIV capsid inhibitor currently in clinical evaluation.



Sara Cherry

Pyrimidine Inhibitors Synergize with Nucleoside Analogues to Block SARS-CoV-2

Sara Cherry is a Professor in the Department of Pathology and Laboratory Medicine at the **University of Pennsylvania**, Scientific Director of the High-throughput Screening Core and Director of the Chemogenomic Discovery Program in the School of Medicine. She obtained her BS with Dr. Peter Schultz at Berkeley, her PhD with Dr. David Baltimore at MIT and her postdoctoral fellowship with Dr. Norbert Perrimon. Upon starting her laboratory at Penn she has applied High-throughput Screening technology to discover mechanisms by which emerging viral pathogens hijack cellular machinery while evading defenses. She has identified innate immune mechanisms and cellular interactions between viruses and cells comparing and contrasting viral families. More recently, she has uncovered new insights into the interplay between metabolic regulation, the microbiota and immune defense. Given the recent pandemic, her laboratory has now applied her screening platform to study the emerging coronavirus, SARS-CoV-2 identifying new antivirals active in the respiratory tract.

William Delaney

Discovery and Development of HBV Core Inhibitors for the Treatment of Chronic Hepatitis B Infection

William Delaney, PhD joined **Assembly Bio** as Chief Scientific Officer in 2020. Prior to joining Assembly, he held positions of increasing responsibility over a 20-year period at Gilead Sciences, serving most recently as an Executive Director in the Biology department. While at Gilead, he led the Viral Hepatitis & Herpes Discovery Biology Group, served as the Research Therapeutic Area Head for HBV, and contributed to the development of several marketed products, including Hepsera®, Viread®, and Vemlidy® for HBV and Sovaldi®, Harvoni®, Epclusa®, and Vosevi® for HCV. He earned a BS in Biotechnology from the University of Delaware and a PhD in Cell and Molecular Biology from the Penn State College of Medicine. In addition, he completed a Postdoctoral Fellowship at the Victorian Infectious Diseases Reference Laboratory (VIDRL), Department of Research & Molecular Development.

Leen Delang

Antiviral Strategies for Arboviruses: a 'Buzzing' Role for the Mosquito Vector?

Leen Delang is an Assistant Professor in Virology at the **Rega Institute for Medical Research** in Leuven, Belgium. She received her PhD in Pharmaceutical Sciences from the University of Leuven in 2011, working on new antiviral therapies for the hepatitis C virus. As a postdoctoral researcher she identified new antiviral drugs against the chikungunya virus. In 2016, she was a visiting researcher in the team of Prof. Anna-Bella Failloux at the Pasteur Institute in Paris, where she studied the transmission of antiviral drug-resistant chikungunya viruses by mosquitoes. In 2019, Leen became an independent principle investigator in Leuven. Her research focuses on understanding the interactions between arboviruses, their mosquito vectors and the mammalian host, and on translating this work into new antiviral strategies to reduce arbovirus transmission and disease. Leen is an editor for the Journal of General Virology and a member of the editorial board of Antiviral Research.

Carl W. Dieffenbach

PLENARY SPEAKER

Drug Development for Viruses of Pandemic Potential, Where Are We Now and Where Are We Going?

Carl W. Dieffenbach, PhD is the Director of the Division of AIDS at the **National Institute of Allergy and Infectious Diseases,** part of the **National Institutes of Health**. Under his leadership, the Division supports a global research portfolio to advance biological knowledge of HIV/AIDS, its related co-infections, and co-morbidities. As a result of his leadership on therapeutics and vaccines for HIV, he was asked to lead the Antiviral Program for Pandemics, a whole of government program to advance direct-acting antivirals for SARS-CoV-2 and additional viruses of pandemic potential in March 2021. Dr. Dieffenbach received his bachelor's degree in biochemistry from the University of Maryland in 1976 and his PhD in biophysics from The Johns Hopkins University in 1984.

David Dulin

Mechanochemistry and Drug Targeting of the SARS-CoV-2 Replication-Transcription Complex from a Single Molecule Perspective

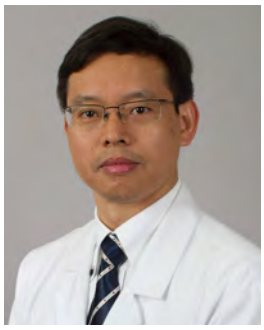
David Dulin is assistant professor at the **Physics and Astronomy department of VU Amsterdam** and junior group leader at the **Medicine Faculty of FAU Erlangen-Nuremberg**. He earned a Bachelor (2004) and a Master of Science (2006) in physics and mathematics at Bordeaux University, including a one-year Erasmus exchange at Bristol University. He earned a PhD at the Charles Fabry Laboratory of the Institut d'Optics and University Paris-Sud, where he developed a single molecule fluorescence microscopy assay to study the mechanism of bacterial and eukaryotic translation elongation dynamics. In 2009, he moved onto his first postdoc at the Bionanosciences Department of TU Delft, where he pioneered high-throughput and high-resolution magnetic tweezers to study viral RNA-dependent RNA polymerases (RdRps) elongation dynamics, nucleotide addition cycle and fidelity. He applied his newly developed assay to characterize the mechanism of action of several nucleotide analogue, discovering that a pyrazine carboxamide incorporation induces long-lived, backtracking pauses in poliovirus elongation dynamics. In 2014, he moved onto a second postdoc at University of Oxford to study bacterial transcription initiation dynamics using single molecule FRET. In 2016, he accepted the Junior Research Group Leader "Physics and Medicine" position at FAU Erlangen-Nuremberg. In 2021, he accepted a second appointment at VU Amsterdam as assistant professor in the Department of Physics and Astronomy. Dulin's research focuses on developing single molecule microscopy techniques to investigate at the single molecular level the mechanism that regulates RNA synthesis and processing in RNA viruses. In particular, his lab has pioneered the study of the coronavirus polymerase nucleotide addition cycle using high-throughput magnetic tweezers, and discovered the mechanism of action of Remdesivir.

Ken Duncan

PLENARY SPEAKER

What Role Can Foundations Play in Driving Innovation Globally in Antiviral Development?

Ken Duncan is Deputy Director of Discovery & Translational Sciences within the Global Health Program of the **Bill & Melinda Gates Foundation**. He leads the foundation's efforts in drug discovery across therapeutic areas currently including TB, malaria, antivirals and contraception. He recently coordinated the drug discovery response to COVID-19 then developed and is implementing a strategy for pandemic preparedness, working together with other funding agencies, to identify safe, effective agents that can be deployed in low-resource settings. He established the TB Drug Accelerator, a new model of public-private collaboration, which aims to discover drug candidates that will contribute to a short-acting TB therapy. He played a major role in launching the Global Health Drug Discovery Institute in Beijing, China. He serves on the Selection Committee of the Global Health Innovative Technology Fund in Japan. Before joining the foundation in 2007, he spent 16 years in the pharmaceutical industry, most recently as Director, Diseases of the Developing World at GlaxoSmithKline. During that time, he helped establish the Global Alliance for TB Drug Development and served on its Scientific Advisory Committee for six years. Dr. Duncan obtained a BSc in Molecular Biology from the University of Edinburgh and a PhD from the University of Glasgow, and completed Postdoctoral Research Fellowships at the University of Glasgow, Massachusetts Institute of Technology and Harvard Medical School.



Pinghui Feng

Explore Viral Infection to Probe Roles of Protein Deamidation

Pinghui Feng is currently a Professor and the Chair of the Section of Infection and Immunity, **Herman Ostrow School of Dentistry, Norris Comprehensive Cancer Center of University of Southern California**. Pinghui obtained his BS from Hunan Normal University, MS from Wuhan Institute of Virology, and PhD from University of Missouri-Kansas City. Before joining University of Southern California, he received postdoctoral training from Dr. Jae Jung's laboratory at Harvard Medical School and established his independent research group at UT Southwestern Medical Center as the Virginia Murchison Linthicum scholar in Medical Research. Pinghui is a recipient of Sustaining Outstanding Achievement in Research award (R35) from NIH.

Pinghui's group has outstanding interest in viral immune evasion strategy, particularly those of human herpesviruses. His lab has discovered that herpesviruses deploy protein deamidation to inactivate innate immune defense. This discovery led to the endeavor to probe the general roles of protein deamidation in fundamental biological processes, such as protein nuclear transport, transcriptional regulation and metabolic reprogramming, in addition to immune defense. Protein deamidation is catalyzed by cellular metabolic enzymes known as glutamine amidotransferases, thus forging potential link between cellular metabolism and other key biological processes. Research in the Feng laboratory was supported by generous startup funds from University of Southern California and grants from private foundations and federal agencies. Pinghui is an Associate Editor for PLoS Pathogens and an editorial member for Journal of Virology. He is a standing member of the advisory committee of International Herpesvirus Workshop and International Workshop on KSHV and Related Agents.

George F. Gao

Monoclonal Antibodies Against Both Flu and RSV

George F. Gao, is a member (academician) of Chinese Academy of Sciences, an international member of National Academy of Sciences, and fellow of African Academy of Sciences, Director-General of Chinese Center for Disease Control and Prevention, and Director and Professor of CAS Key Laboratory of Pathogen Microbiology and Immunology, **Institute of Microbiology, Chinese Academy of Sciences**.

Professor Gao obtained his PhD (DPhil) degree from Oxford University, UK and did his postdoc work in both Oxford University and Harvard University (with a brief stay in Calgary University). His research interests include enveloped viruses and molecular immunology. His group research is mainly focusing on the enveloped virus entry and release, esp. influenza virus interspecies transmission (host jump), structure-based drug-design and structural immunology. He is also interested in virus ecology, especially the relationship between influenza virus and migratory birds or live poultry markets and the bat-derived virus ecology and molecular biology. He has published lots of refereed papers (Including papers in Cell, Nature, Science, The Lancet, New England Journal of Medicine, Proceedings of the National Academy of Sciences USA etc.). His research has recently expanded on public health policy and global health strategy. Gao is a recipient of several international and national awards, including TWAS Medical Prize (2012), Nikkei Asian Prize (Japan 2014), Shulan Medical Sciences Award (2016), the Gamaleya Medal (Russia 2018), HKU Centennial Distinguished Chinese Scholar (2019) and the Qiu Shi Outstanding Scientist and Outstanding Scientific Research Team Awards (2019).

Jeffrey Glenn

Host-Targeting Broad-Spectrum Antivirals for Pandemic Preparedness

Jeffrey Glenn, MD, PhD is a Professor of Medicine (Division of Gastroenterology & Hepatology) and Microbiology & Immunology at **Stanford University School of Medicine**, and the Director of the Center for Hepatitis and Liver Tissue Engineering. He also heads a research laboratory focused on studying molecular virology and the translation of that knowledge into novel antiviral strategies, as well as the development of new treatments for liver diseases and cancer. He is the founder of Eiger BioPharmaceuticals, Inc. (NASDAQ:EIGR), co-founder of Riboscience LLC, and founder of I-Cubed Therapeutics, biotechnology companies developing several new classes of antiviral and anti-cancer drugs.

Glenn was born in Los Angeles and grew up in Switzerland. He received his BA degree in Biochemistry and French Civilization from U.C. Berkeley from where he graduated summa cum laude. He received his MD and PhD in Biochemistry and Biophysics from U.C.S.F. He trained in internal medicine at Stanford University where he completed specialty training in gastroenterology and joined the faculty in 2000.

He is the principal investigator on multiple NIH grants, an inventor on numerous patents, an elected member of the American Society for Clinical Investigation, and a member of the FDA Antiviral Drugs Advisory Committee.

Feng Gu

NITD-688, a Pan-Serotype Inhibitor of the Dengue Virus NS4B Protein, Shows Favorable Pharmacokinetics and Efficacy in Preclinical Animal Models

Feng Gu is Head of Global Program Management and Partnership at **Novartis Institute for Tropical Diseases (NITD)**. NITD is dedicated to discover and develop drugs for neglected tropical diseases. She received undergraduate and graduate trainings in biochemistry in University of Geneva and carried out postdoc research in cell biology and cell signaling in Vollum Institute in the US and McGill Cancer Center in Canada before joining NITD as a research investigator. In NITD, she spent eight years in dengue drug discovery and carried out dengue cell based screens and host target research. She is leading the NITD688 development project team and is also the Head of Global Program Management and Partnership at NITD.

Amy Hartman

New Insights into Cellular Infection by the Mosquito-Transmitted Rift Valley Fever Virus

Dr. Amy Hartman is an Associate Professor in the Center for Vaccine Research and the Department of Infectious Diseases and Microbiology in the **Pitt Graduate School of Public Health**. She performed post-doctoral work in the Special Pathogens Branch at the Centers for Disease Control (CDC) in Atlanta where she studied the pathogenesis of Ebola and other hemorrhagic fever viruses. While at CDC, she was deployed to Angola in 2005 as part of an international response team for the largest Marburg Hemorrhagic Fever outbreak on record. In 2008, she returned to the University of Pittsburgh Regional Biocontainment Lab, a large high containment laboratory designed for the study of pathogenic bacteria and viruses. She uses her expertise with high hazard viruses to ask questions about the basic pathogenesis of these viruses and to perform preclinical evaluation of novel broad-spectrum therapeutic drugs and vaccines in animal models. The focus of her lab is currently on understudied disease outcomes caused by mosquito-borne bunyaviruses, with particular emphasis on Rift Valley fever virus.

Rolf Hilgenfeld

The SARS-CoV-2 Main Protease: New Inhibitors, Crystal Structures, and Mutations

Professor Rolf Hilgenfeld was the Director of the Institute of Biochemistry at the **University of Lübeck, Germany**, from 2003 to 2020. Since May 2020, he has held a Senior Professorship at the **Institute of Molecular Medicine** of the same university. Following his PhD in chemistry and macromolecular crystallography at the Free University of Berlin and postdoctoral training at the Biocenter in Basel, he joined the pharmaceutical company Hoechst AG in Frankfurt, where he established protein crystallography and structure-based drug design. Here he worked on inhibitors of HIV protease and elongation factor Tu. He led the design of a long-acting insulin, which has now annual sales of around 3 billion US$ (2015: 7 billion US$) under the name LantusÒ. In 1995, he accepted the chair of Structural Biochemistry at the University of Jena, where he was Director of the Institute of Molecular Biotechnology from 1998 to 2000. Following his move to Lübeck in 2003, he determined the first crystal structure of any coronavirus protein, that of the main protease, and designed early inhibitor leads against the SARS virus. Later, his group published the crystal structure of the Zika virus protease and more recently, that of the SARS-CoV-2 main protease. His research group follows an integrated approach towards antiviral drug discovery, which includes X-ray crystallography, drug design, and chemical synthesis of inhibitors. In 2009, Rolf Hilgenfeld was awarded an honorary doctorate from the University of South Bohemia, Budweis (Czech Republic), and from 2010 to 2012, he was a Chinese Academy of Sciences Visiting Professor with a co-affiliation at the Shanghai Institute of Materia Medica. In 2015, he received the Ge Hong Medal of the Wuhan Institute of Virology. The focus of his present research is on the structure-based design and chemical synthesis of coronavirus and enterovirus protease inhibitors.

Shan-Lu Liu

Cell-to-Cell Transmission by Emerging Viruses: Mechanisms of Action and Evasion of Host Immunity

Dr. Shan-Lu Liu is a Professor of Virology and Co-Director of the Viruses and Emerging Pathogens' Program of **The Ohio State University's Infectious Diseases Institute**. He obtained his PhD from the University of Washington and Fred Hutchinson Cancer Research Center in Seattle before being recruited to McGill University where he was named as Canada Research Chair. Dr. Liu's research is focused on virus-host interaction, particularly host factors that modulate virus entry and release. In the last few years, research from Dr. Liu's lab has provided insights for understanding how some host restriction factors IFITM, TIM and SERINC limit infection by HIV, Ebolavirus, Zika virus, and SARS-CoV-2, as well as how viruses have evolved antagonism to counteract the host restriction. Dr. Liu is an elected Fellow of American Academy of Microbiology (AAM) and the American Association for the Advancement of Science (AAAS).

Anne Moscona

Inhibiting Entry of Human Paramyxoviruses

Anne Moscona is the Sherie L. Morrison Professor of Microbiology and Immunology, Professor of Pediatrics, and Professor of Physiology and Cellular Biophysics; Director, Center for Host-Pathogen Interaction, at **Columbia University Vagelos College of Physicians and Surgeons**. She is a pediatrician-scientist bridging basic virology with infectious diseases. Her research is focused on basic research on paramyxoviruses that cause serious and prevalent childhood diseases, and on newly emerging paramyxoviruses that affect humans. The focus of her team's work is on the mechanisms of viral entry into host cells in the initial stages of infection, and strategies for interfering with entry and infection. The human parainfluenza viruses, a consistent focus of her study, are an important cause of croup, pneumonia and infant bronchiolitis, major causes of disease and death in infants and in children under 5 years of age. The laboratory is best known for identifying critical roles of the viral receptor binding protein in activating the viral fusion process during infection. By identifying the mechanism of fusion activation, Dr. Moscona and her colleagues identified potential targets for interfering with the viral entry process of these and other enveloped respiratory viruses. She is a Fellow of the American Academy of Microbiology; Member of the American Society of Clinical Investigation (ASCI); Alpha Omega Alpha Medical Honor Society; Society for Pediatric Research, and is currently the Councilor for Medical Virology, American Society for Virology.

Lisa Oestereich

Antiviral Therapy for Lassa Fever

Lisa Oestereich studied Biochemistry at the Medical School Hanover, Germany and obtained her Diploma in 2014. She started her PhD in the Department of Virology of the **Bernhard Nocht Institute for Tropical Medicine**, focusing on the development of mouse models and antiviral therapy for haemorrhagic fever viruses. During her PhD, she could show that the nucleoside analogue Favipiravir is a potent antiviral for the treatment of Crimean-Congo hemorrhagic Fever virus, Ebola virus and Lassa virus infections. From 2015 to 2018, she worked as a postdoctoral fellow and project leader in the Department of Virology. She continued her work with a focus on examining the murine immune response after Lassa Virus infections and started to evaluate drugs that modify the host response as Lassa Fever treatment options. As part of a DFG-funded collaborative research project from BNITM and the Irrua Specialist Teaching Hospital, she analyzed samples from Lassa Fever patients to identify biomarkers for severe Lassa Fever. Since 2019 she has been leading a Leibniz Junior Research Group at BNITM, focusing on analyzing the pathomechanism of Lassa Fever in humans and mice. Her research aims to unravel the host responses, especially of the immune system, to better understand potential targets for host-directed therapeutic approaches.

Kathie Seley-Radtke

2020 ANTONÍN HOLÝ AWARD WINNER

Fleximers – A Strategic Approach to Broad-Spectrum Antiviral Therapeutics

Dr. Kathie Seley-Radtke is a Professor in the Department of Chemistry & Biochemistry at the **University of Maryland, Baltimore County (UMBC)**. Her medicinal chemistry research focuses on targeting coronaviruses including SARS-CoV-2 and MERS, as well as Ebola, Dengue, Yellow Fever viruses, among other infectious diseases using her nucleoside "fleximers". Dr. Seley-Radtke was the 2015-2018 UMBC Presidential Research Professor, as well as the University of Maryland's System-wide Regents Professor for Creativity in Research. In 2016 she was named Maryland Chemist of the Year by the American Chemical Society for her outstanding accomplishments and demonstrated service to the antiviral and medicinal chemistry fields, and most recently, was selected as one of the Baltimore Sun's 25 Women to Watch. Dr. Seley-Radtke is currently the President-Elect of ISAR, as well as a past President for the International Society for Nucleosides, Nucleotides & Nucleic Acids (IS3NA), and a Co-Chair for the 2023 Gordon Research Conference on Nucleosides, Nucleotides & Oligonucleotides.

Marco Vignuzzi

Out of the Box: 20 Years of Targeting Virus Infections Unconventionally

Marco Vignuzzi's lab at the **Institut Pasteur**, founded in 2008, has carved its own niche between the fields of virology and evolutionary sciences. They have since become a mix of experimental evolution lab and computational group. They focus their attention on medically relevant RNA viruses. Their study models include several picornaviruses (EVA71, EVD68, polio, Coxsackievirus, rhinovirus); the alphaviruses (chikungunya, O'nyong'nyong, Sindbis, Mayaro); the flaviviruses (Zika, dengue, West Nile, yellow fever, Usutu); influenza A virus and Coronavirus-SARS-2. They study the population dynamics and evolution of these viruses in animal models (mostly lab mice and wild mosquitoes, but also Zebrafish and Drosophila). They have partnerships with labs in every tropical region of the world with whom they tackle projects in field settings. The lab develops new tools in data visualization and multi-dimensional scaling to study virus infection in lab and clinical settings. Finally, they combine experimental evolution and computation to generate new antiviral approaches that are of interest to both public health and industry.

Fabien Zoulim

Clinical Update on Viral RNA Targeting Agents for Chronic Hepatitis B

Fabien Zoulim obtained his MD in Gastroenterology and Hepatology in Lyon Medical School in 1991. He has also obtained a PhD in Molecular and Cellular Biology and was trained as a post-doctoral researcher at Fox Chase Cancer Center in Philadelphia. He is Professor of Medicine at Lyon I University since 1997. He is Head of the Hepatology Department at the **Hospices Civils de Lyon**, and Head of the Viral Hepatitis Research Laboratory of **INSERM Unit 1052**. Dr Zoulim is currently Associate Editor for Gut. He also served as a Governing Board member of the European Association for the Study of the Liver (EASL). Dr Zoulim received the William Prusoff award of the International Society for Antiviral Research. He is currently coordinating the ANRS "HBV cure" Task Force in France and the "IP-cure-B" project within the EU H2020 workprogram. He co-founded the International Coalition to Eliminate HBV (ICE-HBV: http//:www.ice-hbv.org). He has published more than 500 articles (Web of Science H index 84).

MONDAY, MARCH 21st, 2022

12:00 PM – 1:45 PM

Special Event: Women in Science Roundtable

(In-Person Event Only)

MADISON BALLROOM
(2nd floor)

Chaired by
Rhonda Cardin

2:00 PM – 4:15 PM

Opening Session and Plenary Speakers

COURTYARD BALLROOM

Chaired by
Kara Carter AND **Kathie Seley-Radtke**

2:15 PM

001. **What Role Can Foundations Play in Driving Innovation Globally in Antiviral Development?**

Ken Duncan, Ph.D.[1]
[1]*Bill & Melinda Gates Foundation, Seattle, Washington, United States of America*

3:15 PM

002. **Drug Development for Viruses of Pandemic Potential, Where Are We Now and Where Are We Going?**

Carl Dieffenbach, Ph.D.[1]
[1]*National Institute of Allergy and Infectious Diseases, Division of AIDS, Rockville, Maryland, United States of America*

4:15 PM – 4:30 PM

Break

COURTYARD FOYER

4:30 PM – 5:30 PM

Gertrude Elion Memorial Award Lecture

COURTYARD BALLROOM

Chaired by
Kara Carter AND **Kathie Seley-Radtke**

003. **Developing an Oral Antiviral Agent in a Pandemic: The Evolution of Molnupiravir (EIDD-2801, MK-4482) as a Treatment for COVID-19**

George Painter, Ph.D.[1], Gregory Bluemling, Ph.D.[2], Alexander Kolyhalov, Ph.D.[2], Michael Natchus, Ph.D.[3]
[1]EIDD, DRIVE, Department of Pharmacology and Chemical Biology, Atlanta, Georgia, United States of America; [2]EIDD, DRIVE, Atlanta, Georgia, United States of America; [3]EIDD, Atlanta, Georgia, United States of America

5:30 PM – 6:30 PM

Opening Reception

(In-Person Event Only)

MADISON BALLROOM
(2nd floor)

TUESDAY, MARCH 22nd, 2022

8:30 AM – 9:15 AM

Antonín Holý Memorial Award Lecture

COURTYARD BALLROOM

Chaired by
Kathie Seley-Radtke AND **Andrea Brancale**

004. **Structure-guided Antiviral Drug Discovery – A Tale of Two Viruses**

Mark von Itzstein, Ph.D.[1]
[1]Institute for Glycomics, Griffith University, Gold Coast, Queensland, Australia

9:15 AM – 11:00 AM

Non-coronavirus Respiratory Viruses Session

COURTYARD BALLROOM

Chaired by
Kathie Seley-Radtke AND **Mark von Itzstein**

9:15 AM

005. **Inhibiting Entry of Human Paramyxoviruses**

Anne Moscona, M.D.[1]
[1]Columbia University Vagelos College of Physicians and Surgeons, New York, New York, United States of America

9:45 AM

006. **Inducing Phase Transition in Viral Condensates. A Novel Antiviral Strategy**

Ralf Altmeyer, Ph.D.[1], Jennifer Risso-Ballester, Ph.D.[2], Marie Galloux, Ph.D.[3], Ronan Le Goffic, Ph.D.[3], Charles-Adrien Richard, Ph.D.[3], Fortune Hontonnou, Ph.D.[3], Jean-François Eléouët, Ph.D.[3], Marie-Anne Rameix-Welti, M.D., Ph.D.[2]

[1]Medusa Therapeutics, Hong Kong, Hong Kong SAR, China; [2]Université Paris-Saclay, INSERM, Université de Versailles St. Quentin, UMR 1173 (2I), Versailles, France; [3]Université Paris-Saclay, INRAE, Unité de Virologie et Immunologie Moléculaires (UR892), Jouy-en-Josas, France

10:30 AM

008. **Treatment of EV-D68 Respiratory and Neurological Disease in AG129 Mice with a Monoclonal Antibody, EV68-228**

Brett Hurst, Ph.D.[1], Matthew Vogt, M.D., Ph.D.[2], Bart Tarbet, Ph.D.[1], James Crowe, M.D.[3]

[1]Utah State University, Logan, United States, United States of America; [2]University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, North Carolina, United States of America; [3]Vanderbilt University Medical Center, Nashville, Tennessee, United States of America

10:40 AM

009. **Impact of PA E23G/K Substitutions on Influenza A Virus Fitness and Baloxavir Susceptibility**

Jeremy Jones, Ph.D.[1], Philippe Pascua, Ph.D.[1], Richard Webby, Ph.D.[1], Elena Govorkova, M.D., Ph.D.[1]

[1]St. Jude Children's Research Hospital, Memphis, Tennessee, United States of America

10:15 AM – 10:30 AM

Break

COURTYARD FOYER

11:00 AM – 12:00 PM

PechaKucha Competition

COURTYARD BALLROOM

Chaired by
Kathie Seley-Radtke

12:00 PM – 1:30 PM

Lunch

(on your own)

1:30 PM – 2:15 PM

William Prusoff Memorial Award Lecture

COURTYARD BALLROOM

Chaired by
Kara Carter AND **Kathie Seley-Radtke**

010. **Targeted Protein Degradation as an Antiviral Strategy**
Priscilla Yang, Ph.D.[1]
[1]*Stanford University School of Medicine, Stanford, California, United States of America*

2:15 PM – 5:00 PM

Retroviruses and Other Viruses Session

COURTYARD BALLROOM

Chaired by
Chris Meier AND **Brian Gentry**

2:15 PM
011. **Cell-to-Cell Transmission by Emerging Viruses: Mechanisms of Action and Evasion of Host Immunity**
Shan-Lu Liu, M.D., Ph.D.[1]
[1]*The Ohio State University, Columbus, Ohio, United States of America*

2:45 PM
012. **Lenacapavir: The First Clinically Active Long-Acting Inhibitor of HIV Capsid**
Christian Callebaut, Ph.D.[1]
[1]*Gilead Sciences, Inc., Foster City, California, United States of America*

3:15 PM
013. **Antiviral Therapy for Lassa Fever**
Elisa Pallasch, B.S.[1], Sabrina Bockholt, B.S.[1], Jonas Müller, B.S.[1], Stephan Günther, M.D., Ph.D.[1], **Lisa Oestereich, Ph.D.**[1]
[1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

4:00 PM
014. **Prophylactic Treatment with a Defective Interfering Particle-Based Therapeutic Protects Hamsters from Lethal Nipah Virus Disease Primarily by Direct Inhibition Mechanisms**
Stephen Welch, Ph.D.[1], Jessica Spengler, Ph.D.[1], Jessica Harmon, M.S.[1], JoAnn Coleman-McCray, B.S.[1], Sarah Genzer, D.V.M.[1], Teresa Sorvillo, Ph.D.[1], Florine Scholte, Ph.D.[1], Michael Lo, Ph.D.[1], Joel Montgomery, Ph.D.[1], Stuart Nichol, Ph.D.[1], Christina Spiropoulou, Ph.D.[1]
[1]*Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America*

4:10 PM

015. **Small Molecule Antivirals Inhibit HuNoV GII.4 in Human Intestinal Enteroids**

Nanci Santos-Ferreira, M.S.[1], Carmen Mirabelli, Ph.D.[2], Jonathan Sexton, Ph.D.[3], Johan Neyts, Ph.D.[1], Christiane Wobus, Ph.D.[2], Joana Rocha-Pereira, Ph.D.[1]

[1]KU Leuven, Rega Institute, Laboratory of Virology & Chemotherapy, Leuven, Belgium, Belgium; [2]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, United States of America; [3]Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan, Michigan, United States of America

4:20 PM

007V. **Monoclonal Antibodies against both Flu and RSV**

George F. Gao, Ph.D.[1]

[1]Institute of Microbiology, Chinese Academy of Sciences, Beijing, China

3:45 PM – 4:00 PM

Break

COURTYARD FOYER

5:00 PM – 7:00 PM

Poster Session 1

(In-Person Event Only)

COMPASS ROOMS
(3rd floor)

Light refreshments provided

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 44.

WEDNESDAY, MARCH 23rd, 2022

8:30 AM – 9:15 AM

Antonín Holý Memorial Award Lecture (2020)

COURTYARD BALLROOM

Chaired by
Kara Carter AND **Chris Meier**

016. **Fleximers – A Strategic Approach to Broad-spectrum Antiviral Therapeutics**
Kathie Seley-Radtke, Ph.D.[1]
[1]*University of Maryland, Baltimore County, Baltimore, Maryland, United States of America*

9:15 AM – 12:15 PM

Broad Spectrum Antiviral Drugs and Pandemic Preparedness Session

COURTYARD BALLROOM

Chaired by
John Bilello AND **Priscilla Yang**

9:15 AM
017. **Out of the Box: 20 Years of Targeting Virus Infections Unconventionally**
Marco Vignuzzi, Ph.D.[1]
[1]*Infectious Diseases Labs ID Labs, A*Star, Singapore*

9:45 AM
018V. **The SARS-CoV-2 Main Protease: New Inhibitors, Crystal Structures, and Mutations**
Rolf Hilgenfeld, Ph.D.[1], Kaixuan Zhang, M.S.[1], Judith Roeske, M.S.[1], Linlin Zhang, Ph.D.[1], Matthias Goehl, Ph.D.[2], Mark Broenstrup, Ph.D.[2], Katharina Rox, Ph.D.[2], Yuri Kusov, Ph.D.[1], Ravikumar Akula, Ph.D.[1], Haifa El Kilani, Ph.D.[1], Mohamed Ibrahim, Ph.D.[1], Xinyuanyuan Sun, M.S.[1]
[1]*University of Luebeck, Institute of Molecular Medicine, Luebeck, Germany;* [2]*Helmholtz Centre for Infection Research, Dept. of Chemical Biology, Braunschweig, Germany*

10:30 AM
019. **Host-targeting Broad-spectrum Antivirals for Pandemic Preparedness**
Jeffrey Glenn, M.D., Ph.D.[1]
[1]*Stanford University School of Medicine, Stanford, California, United States of America*

11:00 AM
020. **Efficient Incorporation and Template-Dependent Polymerase Inhibition are Major Determinants for the Broad-Spectrum Antiviral Activity of Remdesivir**
Calvin Gordon, Ph.D.[1], Hery Lee, Ph.D.[1], Egor Tchesnokov, Ph.D.[1], Jason Perry, Ph.D.[2], Joy Feng, Ph.D.[2], John Bilello, Ph.D.[2], Danielle Porter, Ph.D.[2], Matthias Götte, Ph.D.[1]
[1]*Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada;* [4]*Gilead Sciences, Inc., Foster City, California, United States of America*

11:10 AM

021. Countering Pathogenic New World Mammarenavirus Infections Through Receptor-Targeted Disruption of Virus Entry

Brady Hickerson, Ph.D.[1], Cristian Payes, Ph.D.[2], Lars Clark, Ph.D.[3], Kevin Bailey, B.S.[1], Eric Sefing, M.S.[1], Samantha Zink, Ph.D.[4], James Ziegenbein, B.S.[4], Pierre Candelaria, Ph.D.[5], Jonathan Abraham, M.D., Ph.D.[3], Tracy Daniels-Wells, Ph.D.[5], Gustavo Helguera, Ph.D.[2], Manuel Penichet, M.D., Ph.D.[5], **Brian Gowen, Ph.D.[1]**

[1]Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States of America; [2]Instituto de Biología y Medicina Experimental (IBYME CONICET), Buenes Aires, Argentina; [3]Department of Microbiology, Blavatnik Institute, Harvard Medical School, Boston, Massachusetts, United States of America; [4]Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, California, United States of America; [5]Division of Surgical Oncology, Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California, United States of America

11:20 AM

022. Development of Small Molecule Entry Inhibitors as Potential Filoviral Therapeutics

Laura Cooper, B.S.[1], Adam Schafer, M.D., Ph.D.[1], Rui Xiong, Ph.D.[1], Gregory Thatcher, Ph.D.[1], Lijun Rong, Ph.D.[1]

[1]University of Illinois at Chicago, Chicago, Illinois, United States, United States of America

11:30 AM

023. A Photoactivable Chlorophyll-Derived Product with Broad Antiviral Activity against Enveloped Viruses Including Highly Pathogenic Coronaviruses

Thomas Meunier, Ph.D.[1], Lowiese Desmarets, Ph.D.[1], Simon Bordage, Ph.D.[2], Moussa Bamba, Ph.D.[2], Kevin Hervouet, Ph.D.[1], Yves Rouille, Ph.D.[1], Nathan Francois, B.S.[1], Marion Decossas, M.D.[3], Valentin Sencio, Ph.D.[1], Francois Trottein, Ph.D.[1], Fezan Tra Bi, Ph.D.[4], Olivier Lambert, Ph.D.[3], Jean Dubuisson, Ph.D.[1], Sandrine Belouzard, Ph.D.[1], Sevser Sahpaz, Ph.D.[2], **Karin Seron, Ph.D.[1]**

[1]Center for Infection and Immunity of Lille, Lille, France; [2]BioEcoAgro, Lille, France; [3]University Bordeaux, Bordeaux, France; [4]Université Nangui Abrogoua, Abidjan, Côte d'Ivoire

11:40 AM

024. A Broad-Spectrum Ribonucleoside Analog, EIDD-2749, Provides Protection Against Enterovirus D68 and 71 Infections in Mouse Models

E. Bart Tarbet, Ph.D.[1], Brett Hurst, Ph.D.[1], Manohar Saindane, Ph.D.[2], Alexander Kolykhalov, M.D., Ph.D.[2], George Painter, Ph.D.[2], Gregory Bluemling, Ph.D.[2]

[1]Utah State University, Logan, United States of America; [2]Emory University, Atlanta, Georgia, United States of America

11:50 AM

025. Antiviral Activity of Geneticin Against SARS-CoV-2

Gregory Mathez, M.S.[1], Carmine Varricchio, Ph.D.[2], Caroline Tapparel, Ph.D.[3], Laurent Kaiser, M.D.[4], Andrea Brancale, Ph.D.[2], Valeria Cagno, Ph.D.[1]

[1]Institute of Microbiology, University Hospital of Lausanne and University of Lausanne, Switzerland, Lausanne, Vaud, Switzerland; [2]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK, Cardiff, United Kingdom of Great Britain and Northern Ireland; [3]Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland, Geneva, Switzerland; [4]Geneva University Hospitals, Infectious Diseases Divisions, Geneva, Switzerland, Geneva, Switzerland

12:00 PM

026. **Automated Brightfield Microscopy and AI to Develop Rapid High Throughput Infectivity Assays for Screening Antiviral Drugs and Monitoring Vaccine Effectiveness against Emerging Variants**

Rupert Dodkins, Ph.D.[1], Tess Overton, B.S.[2], John Delaney, M.S.[1], Kathy Yeung, M.B.A.[1], Frank Scholle, Ph.D.[2], **Ilya Goldberg, Ph.D.[1]**

[1]ViQi Inc, Santa Barbara, California, United States of America; [2]NC State University, Raleigh, North Carolina, United States of America

10:15 AM – 10:30 AM

Break

COURTYARD FOYER

12:15 PM – 1:00 PM

Late-breaking Oral Presentations

COURTYARD BALLROOM

Chaired by

Luis Schang AND **Jerome Deval**

12:15 PM

060. **Intermittent Therapy with Helicase-Primase Drug Candidate IM-250 Reduces Reactivation Competency of Latent Neural Herpes Simplex Virus Infections**

Gerald Kleymann, Ph.D.[1], Christian Gege, Ph.D.[1], Fernando J. Bravo, Ph.D.[2], David I. Bernstein, Ph.D.[2], Nancy M. Sawtell, Ph.D.[2]

[1]Innovative Molecules GmbH, Munich, Bavaria, Germany; [2]Children's Hospital Medical Center CCHMC, Cincinnati, Ohio, United States of America; [3]University of Cincinnati College of Medicine, Cincinnati, Ohio, United States of America

12:25 PM

061. **A Bifunctional Immune Modulator Exhibits Potent Antiviral Activity in HBV Infection Models**

Antoine, Alam, Ph.D.[1], Xavier Marniquet, B.S.[1], Marion Dajon, Ph.D. [1], Julie Montegut, B.S.[1], Odile Bonnin, B.S.[1], Charlotte Blanc, B.S. [1], Christelle Marcou, B.S. [1], Juliette Lavaux, Ph.D. [1],Michel Didier, Ph.D.[2], Franck Augé, Ph.D. [2], Yaligara Veeranagouda, Ph.D. [2], Galina Boldina, Ph.D. [2], Celine Lemoine, Ph.D. [2], Jacques Duams, Ph.D. [2], Thomas Bouquin, Ph.D. [2], Annabelle Milla, Ph.D.[1], Hugh Watson, Ph.D. [1], Kara Carter, Ph.D.[3]

[1]Evotec, Lyon, France; [2]Sanofi, Paris, France; [3]Evotec, Saco, ME, United States of America

12:35 PM

062. The Nucleoside Analog Antiviral CMX521 Inhibits SARS-CoV-2 in Human Airway Epithelial Cell Cultures and Exhibits Prophylactic and Therapeutic Efficacy Against Respiratory Disease in a Mouse Model of SARS-CoV-2 Infection

Randall Lanier, Ph.D.[1], Mark T. Heise, Ph.D. [2], Victoria K. Baxter, D.V.M., Ph.D.[2], Sharon Taft-Benz, Ph.D. [2], Audrey C. Knight, Ph.D. [2], Elizabeth J. Anderson, M.S. [2], Amanda P. Schauer, Ph.D. [2], Rebekah Dickmander, B.S. [2], Mohammed Kabir, Ph.D. [1], John A. Dunn, Ph.D. [1], Phiroze Sethna, Ph.D. [1], Venkat Lakshmanan, Ph.D. [1], Heidi M. Colton, M.S. [1], Nathaniel J. Moorman, Ph.D. [2]

[1]Chimerix, Durham, North Carolina, United States of America; [2]UNC-Chapel Hill, Chapel Hill, North Carolina, United States of America

12:45 PM

063. Structural and Mechanistic Characterization of Non-Neutralizing Antibodies Targeting Crimean-Congo Hemorrhagic Fever

Ian Durie, Ph.D. Seeking[1], Elif Karaaslan, Ph.D.[2], Jessica R. Spengler, D.V.M., Ph.D.[3], Joseph W. Golden, Ph.D.[4], Jack McGuire, M.S.[2], Christina F. Spiropoulou, Ph.D.[3], Zahra R. Tehrani, Ph.D.[5], Iftihar Koksal, M.D.[6], Gurdal Yilmaz=, M.D.[7], Hanife Nur Karakoc, M.D.[8], Sanaz Hamidi, M.S.[7], Cansu Albay, M.S. [7], Aura R. Garrison, Ph.D.[4], Mohammad M. Sajadi, M.D.[5], Eric Bergeron, Ph.D.[3], Scott D. Pegan, Ph.D.[2]

[1]University of Georgia, Athens, Georgia, United States of America; [2]University of California Riverside, Riverside, California, United States of America; [3]Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America; [4]United States Army Medical Research Institute of Infectious Diseases, Detrick, Maryland, United States of Amerca; [5]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, United States of America; [6]Department of Infectious Disease and Clinical Microbiology, Acibadem University, Atakent Hospital, Istanbul, Turkey; [7]Department of Infectious Diseases, Karadeniz Technical University School of Medicine, Trabzon, Turkey; [8]Department of Infectious Disease and Clinical Microbiology, Bitlis State Hospital, Bitlis, Turkey

1:00 – 3:00 PM

Poster Session 2

(In-Person Event Only)

COMPASS ROOMS
(3rd floor)

Lunch provided

1:00 – 2:00 PM
EVEN numbered poster presentations

2:00 – 3:00 PM
ODD numbered poster presentations

All posters are listed in numerical order starting on page 44.

THURSDAY, MARCH 24th, 2022

8:30 AM – 9:15 AM

Women in Science Award Lecture

COURTYARD BALLROOM

Chaired by
Rhonda Cardin AND **Jennifer Moffat**

027. **Fighting Viral Hemorrhagic Fevers: From the Benches to the Trenches**
Christina Spiropoulou, Ph.D.[1]
[1]*Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America*

9:15 AM – 12:00 PM

Arboviruses Session

COURTYARD BALLROOM

Chaired by
Andrea Brancale AND **Justin Julander**

9:15 AM
028. **Antiviral Strategies for Arboviruses: A 'Buzzing' Role for the Mosquito Vector?**
Leen Delang, Ph.D.[1]
[1]*University of Leuven, Leuven, Belgium*

9:45 AM
029. **New Insights into Cellular Infection by the Mosquito-Transmitted Rift Valley Fever Virus**
Amy Hartman, Ph.D.[1]
[1]*University of Pittsburgh, Pittsburgh, Pennsylvania. United States of America*

10:30 AM
030. **NITD-688, A Pan-Serotype Inhibitor of the Dengue Virus NS4B Protein, Shows Favorable Pharmacokinetics and Efficacy in Preclinical Animal Models**
Feng Gu, Ph.D.[1]
[1]*Novartis Institute for Tropical Diseases, Emeryville, California, United States of America*

11:00 AM
031. **AI-Derived Antibody Discovery – Humanoids for Global Good**
Randal Ketchem, Ph.D.[1]
[1]*Just - Evotec Biologics, Seattle, Washington, United States of America*

11:10 AM

032. Dengue Virus Infection and Dissemination in Aedes Mosquitoes is Significantly Reduced Upon Exposure to JNJ-A07, A Potent DENV Inhibitor, In The Blood Meal

Ana Rosales Rosas, M.S.[1], Lanjiao Wang, Ph.D.[1], Suzanne Kaptein, Ph.D.[1], Olivia Goethals, Ph.D.[2], Leen Delang, Ph.D.[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium; [2]Janssen Global Public Health, Janssen Pharmaceutica, Beerse, Belgium

11:20 AM

033. AT-752, A Double Prodrug of a Guanosine Nucleotide Analog, is Effective Against Yellow Fever Virus in a Hamster Model.

Abbie Weight, B.S.[1], Kai Lin, Ph.D.[2], Steven Good, M.S.[2], Adel Moussa, Ph.D.[2], Jean-Pierre Sommadossi, Ph.D.[2], Justin Julander, Ph.D.[1]

[1]Institute for Antiviral Research, Utah State University, Logan, Utah, United States of America; [2]Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States of America

11:30 AM

034. Guanine Quadruplexes in the RNA Genome of the Tick-borne Encephalitis Virus: A New Antiviral Target

Ludek Eyer, Ph.D.[1], Jiri Holoubek, Ph.D. Seeking[1], Daniel Ruzek, Ph.D.[1], Daniel Renciuk, Ph.D.[2]

[1]Veterinary Research Institute, Brno, Czechia; [2]Institute of Biophysics of the Czech Academy of Sciences, Brno, Czechia

11:40 AM

035. In Situ Click Chemistry Applied to Bunyavirales: From Conventional Drug Design to Enzymes Assembling Their Own Inhibitors like LEGOs®

Laura Garlatti, M.S.[1], Mikael Feracci, Ph.D.[1], Sergio Hernandez, Ph.D.[1], Bruno Canard, Ph.D.[1], Juan Reguera, Ph.D.[1], François Ferron, Ph.D.[1], Karine Alvarez, Ph.D.[1]

[1]Aix-Marseille Université, Marseille, Bouches-Du-Rhône, France

10:15 AM – 10:30 AM

Break

COURTYARD FOYER

12:00 PM – 12:15 PM

ISAR Annual Business Meeting

COURTYARD BALLROOM

PRESIDENT: Kara Carter

TREASURER: Brian Gowen

SECRETARY: Jinhong Chang

12:15 PM – 2:00 PM

Lunch

(on your own)

2:00 PM – 2:45 PM

Diversity Speaker Award Lecture

COURTYARD BALLROOM

Chaired by
Craig Cameron AND **Kara Carter**

036. **The COVID-19 Pandemic: A View from the Bench**
J. Victor Garcia-Martinez, Ph.D.[1]
[1]*University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America*

2:45 PM – 5:45 PM

Coronaviruses Session

COURTYARD BALLROOM

Chaired by
Craig Cameron AND **Kara Carter**

2:45 PM
037. **Pyrimidine Inhibitors Synergize with Nucleoside Analogues to Block SARS-CoV-2**
Sara Cherry, Ph.D.[1]
[1]*University of Pennsylvania, Philadelphia, Pennsylvania, United States of America*

3:15 PM
038V. **Mechanochemistry and Drug Targeting of the SARS-CoV-2 Replication-transcription Complex from a Single Molecule Perspective**
David Dulin, Ph.D.[1]
[1]*VU Amsterdam & FAU Erlangen-Nuremberg, Amsterdam, Netherlands*

4:00 PM
039. **Profile of PBI-0451 an Orally Administered 3CL Protease Inhibitor of SARS-CoV-2 for COVID-19**
Lee Arnold, Ph.D.[1], **Uri Lopatin**[2]
[1]*Pardes Biosciences, Inc., Carlsbad, California, United States of America;* [2]*Pardes Biosciences, Inc., Maryland, United States of America*

4:30 PM
040. **Strategies to Interfere with Nucleotide Excision by the 3′-to-5′ Exoribonuclease from SARS-CoV-2**
Jamie Arnold, Ph.D.[1], Rukesh Chinthapatla, B.S.[1], Mohamad Sotoudegan, Ph.D.[1], Craig Cameron, Ph.D.[1]
[1]*The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America*

4:40 PM

041. Efficacy in a SARS-CoV-2 African Green Monkey Model Validates a Prodrug Approach for Oral Delivery of Remdesivir Parent Nucleoside GS-441524

Jared Pitts, Ph.D.[1], Darius Babusis, Ph.D.[1], Diane Lye, Ph.D.[1], Kimberly Barrett, Ph.D.[1], Xianghan Lu, M.S.[1], Meghan Vermillion, D.V.M., Ph.D.[2], Adriana Kajon, Ph.D.[2], Roy Bannister, Ph.D.[1], Raju Subramanian, Ph.D.[1], Danielle Porter, Ph.D.[1], Tomas Cihlar, Ph.D.[1], Richard Mackman, Ph.D.[1], John Bilello, Ph.D.[1]

[1]Gilead Sciences Inc., Foster City, California, United States of America; [2]Lovelace Biomedical, Albuquerque, New Mexico, United States of America

4:50 PM

042. A Dual Mechanism of Action of AT-527 Against SARS-CoV-2 Polymerase

Ashleigh Shannon, Ph.D.[1], Véronique Fattorini, M.S.[1], Bhawna Sama, M.S.[1], Barbara Selisko, Ph.D.[1], Mikael Feracci, Ph.D.[1], Camille Falcou, M.S.[1], Pierre Gauffre, M.S.[1], Adrien Delpal, M.S.[1], Etienne Decroly, Ph.D.[1], Karine Alvarez, Ph.D.[1], Cécilia Eydoux, Ph.D.[1], Jean-Claude Guillemot, Ph.D.[1], Adel Moussa, Ph.D.[2], Steven Good, Ph.D.[2], Kai Lin, Ph.D.[2], Jean-Pierre Sommadossi, Ph.D.[2], Yingxiao Zhu, Ph.D.[3], Xiaodong Yan, Ph.D.[3], Hui Shi, Ph.D.[3], François Ferron, Ph.D.[1], Bruno Canard, Ph.D.[1]

[1]AFMB and CNRS, AIX Marseille Université, Marseille, France; [2]Atea Pharmaceuticals, Inc, Boston, Massachusetts, United States of America; [3]WuxiBiortus Biosciences Co. Ltd., Jiangyin, China

5:00 PM

043. Dual Inhibition of SARS-CoV-2 and Human Rhinovirus with Protease Inhibitors in Clinical Development

Cheng Liu, Ph.D.[1], Sandro Boland, Ph.D.[2], Michael Scholle, Ph.D.[3], Dorothée Bardiot, Ph.D.[2], Arnaud Marchand, Ph.D.[2], Patrick Chaltin, Ph.D.[2], Lawrence M. Blatt, Ph.D.[1], Leonid Beigelman, Ph.D.[1], Julian A. Symons, Ph.D.[1], Pierre Raboisson, Ph.D.[4], Zackary Gurard-Levin, Ph.D.[3], Koen Vandyck, Ph.D.[1], **Jerome Deval, Ph.D.[1]**

[1]Aligos Therapeutics, South San Francisco, California, United States of America; [2]Cistim, Leuven, Belgium; [3]SAMDI Tech, Chicago, Illinois, United States of America; [4]Aligos Therapeutics, Leuven, Belgium

5:10 PM

044. In Vitro Selection and Characterization of a SARS-CoV-2 Isolate Resistant to Remdesivir

Kim Donckers, M.S.[1], Laura Vangeel, Ph.D.[1], Steven De Jonghe, Ph.D.[1], Johan Neyts, Ph.D.[1], **Dirk Jochmans, Ph.D.[1]**

[1]KU Leuven - Rega Institute, Leuven, Belgium

5:20 PM

045. Oral Inhibitors of the SARS-CoV-2 Main Protease for the Treatment of COVID-19

Dafydd Owen, Ph.D.[1]; **Rhonda Cardin, Ph.D.[2]**

[1]Pfizer Worldwide Research Development and Medicine, Cambridge, Massachusetts, United States of America; [2]Pfizer Worldwide Research Development and Medicine, Pearl River, New York, United States of America

5:30 PM

046. **Picomolar Covalent Reversible Inhibitors of CoVs Main Proteases Effectively Inhibit SARS-CoV-2 Replication: Design, Synthesis, Biological Evaluation, and X-Ray Structural Characterization**

Rolando Cannalire, Ph.D.[1], Francesca Esposito, Ph.D.[2], Irina Stefanelli, M.S.[1], Angela Corona, Ph.D.[2], Francesco Di Leva, Ph.D.[1], Emilia Cassese, M.S.[1], Paola Storici, Ph.D.[3], Elisa Costanzi, Ph.D.[3], Enzo Tramontano, Ph.D.[2], **Vincenzo Summa, Ph.D.[1]**

[1]Department of Pharmacy - University of Napoles Federico II, Naples, Italy; [2]Department of Life and Environment Sciences - University of Cagliari, Cagliari, Italy; [3]Elettra Syncroton, Trieste, Italy

3:45 PM – 4:00 PM

Break

COURTYARD FOYER

7:00 PM – 10:00 PM

Closing Dinner Event

(In-Person Event Only)

COLUMBIA TOWER CLUB
701 5TH AVENUE
(2 blocks from hotel)

RSVP Required

FRIDAY, MARCH 25th, 2022

8:30 AM – 9:30 AM

Special Event: Career Development Interactive Workshop

COURTYARD BALLROOM

Chaired by
Leen Delang AND **Brian Gowen**

The Power of Mentoring

Harmit Malik
Fred Hutchinson Cancer Research Center

9:30 AM – 12:00 PM

Hepatitis and Herpes Viruses Session

COURTYARD BALLROOM

Chaired by
Jerome Deval AND **Roger Ptak**

9:30 AM
047V. **Clinical Update on Viral RNA Targeting Agents for Chronic Hepatitis B**
Fabien Zoulim, M.D., Ph.D.[1]
[1]*INSERM, Lyon, France*

10:00 AM
048. **Discovery and Development of HBV Core Inhibitors for the Treatment of Chronic Hepatitis B Infection**
William Delaney, Ph.D.[1]
[1]*Assembly Bio, South San Francisco, California, United States of America*

10:45 AM
049. **Explore Viral Infection to Probe Roles of Protein Deamidation**
Pinghui Feng, Ph.D.[1]
[1]*University of Southern California, Los Angeles, California, United States of America*

11:15 AM
050. **LAVR-289, a New Broadly Active Acyclonucleoside Phosphonate Prodrug is Highly Effective in the SCID-Hu Mouse Model of Varicella Zoster Virus Replication**
Jennifer Moffat, Ph.D.[1], Megan Lloyd, Ph.D.[1], Dongmei Liu, M.S.[1], Vincent Roy, Ph.D.[2], Luigi Agrofoglio, Ph.D.[2]
[1]*SUNY Upstate Medical University, Syracuse, New York, United States of America;* [2]*Universite d'Orleans, Orleans, Loiret, France*

11:25 AM
052. **Characterization of the N-hydroxypyridinediones (HPD) and the N-hydroxynapthyridinones (HNO) as HBV RNase H inhibitors**
Molly Woodson, M.S.[1], Varin Gupta, B.S.[1], Makafui Gasonoo, Ph.D.[2], Sotirios Katsamakas, Ph.D.[3], Marvin Meyers, Ph.D.[2], Grigoris Zoidis, Ph.D.[3], John Tavis, Ph.D.[1]
[1]*Saint Louis University School of Medicine Department of Molecular Microbiology and Immunology, St Louis, United States of America;* [2]*Saint Louis University Department of Chemistry, St Louis, United States of America;* [3]*National and Kapodistrian University of Athens, Department of Pharmacy, Athens, Greece*

11:35 AM

053. Resistance Analysis in a Phase 2 Clinical Trial with the Helicase-Primase Inhibitor Pritelivir in Immunocompromised Adults with Acyclovir Resistant Herpes Simplex Virus (HSV) Infection

Alexander Birkmann, Ph.D.[1], Alexander Greninger, M.D., Ph.D.[2], Meei-Li Huang, Ph.D.[2], Manickam Rangaraju, M.D.[1], Stacy Selke, M.S.[2], Melanie Sumner, M.S.[1], Burkhard Timmler, M.D.[1], Hong Xie, M.S.[2], Haiying Zhu, M.S.[2], Holger Zimmermann, Ph.D.[1], Anna Wald, M.D., MPH[2], Keith Jerome, M.D., Ph.D.[2]

[1]AiCuris Anti-Infective Cures AG, Wuppertal, Germany; [2]Virology Division, Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, United States of America

10:30 AM – 10:45 AM

Break

COURTYARD FOYER

12:00 PM – 12:30 PM

Shotgun Presentations and Closing Remarks

COURTYARD BALLROOM

015.* Small Molecule Antivirals Inhibit HuNoV GII.4 in Human Intestinal Enteroids

Nanci Santos-Ferreira, M.S.[1], Carmen Mirabelli, Ph.D.[2], Jonathan Sexton, Ph.D.[3], Johan Neyts, Ph.D.[1], Christiane Wobus, Ph.D.[2], Joana Rocha-Pereira, Ph.D.[1]

[1]KU Leuven, Rega Institute, Laboratory of Virology & Chemotherapy, Leuven, Belgium, Belgium; [2]Department of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, United States of America; [3]Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan, Michigan, United States of America

020.* Efficient Incorporation and Template-Dependent Polymerase Inhibition are Major Determinants for the Broad-Spectrum Antiviral Activity of Remdesivir

Calvin Gordon, Ph.D.[1], Hery Lee, Ph.D.[1], Egor Tchesnokov, Ph.D.[1], Jason Perry, Ph.D.[2], Joy Feng, Ph.D.[2], John Bilello, Ph.D.[2], Danielle Porter, Ph.D.[2], Matthias Götte, Ph.D.[1]

[1]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada; [4]Gilead Sciences, Inc., Foster City, California, United States of America

025.* Antiviral Activity of Geneticin Against SARS-CoV-2

Gregory Mathez, M.S.[1], Carmine Varricchio, Ph.D.[2], Caroline Tapparel, Ph.D.[3], Laurent Kaiser, M.D.[4], Andrea Brancale, Ph.D.[2], Valeria Cagno, Ph.D.[1]

[1]Institute of Microbiology, University Hospital of Lausanne and University of Lausanne, Switzerland, Lausanne, Vaud, Switzerland; [2]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK, Cardiff, United Kingdom of Great Britain and Northern Ireland; [3]Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland, Geneva, Switzerland; [4]Geneva University Hospitals, Infectious Diseases Divisions, Geneva, Switzerland, Geneva, Switzerland

026.* Automated Brightfield Microscopy and AI to Develop Rapid High Throughput Infectivity Assays for Screening Antiviral Drugs and Monitoring Vaccine Effectiveness against Emerging Variants

Rupert Dodkins, Ph.D.[1], Tess Overton, B.S.[2], John Delaney, M.S.[1], Kathy Yeung, M.B.A.[1], Frank Scholle, Ph.D.[2], **Ilya Goldberg, Ph.D.**[1]

[1]ViQi Inc, Santa Barbara, California, United States of America; [2]NC State University, Raleigh, North Carolina, United States of America

032.* Dengue Virus Infection and Dissemination in Aedes Mosquitoes is Significantly Reduced Upon Exposure to JNJ-A07, A Potent DENV Inhibitor, In The Blood Meal

Ana Rosales Rosas, M.S.[1], Lanjiao Wang, Ph.D.[1], Suzanne Kaptein, Ph.D.[1], Olivia Goethals, Ph.D.[2], Leen Delang, Ph.D.[1]

[1]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium, Leuven, Belgium; [2]Janssen Global Public Health, Janssen Pharmaceutica, Beerse, Belgium

033.* AT-752, A Double Prodrug of a Guanosine Nucleotide Analog, is Effective Against Yellow Fever Virus in a Hamster Model.

Abbie Weight, B.S.[1], Kai Lin, Ph.D.[2], Steven Good, M.S.[2], Adel Moussa, Ph.D.[2], Jean-Pierre Sommadossi, Ph.D.[2], Justin Julander, Ph.D.[1]

[1]Institute for Antiviral Research, Utah State University, Logan, Utah, United States of America; [2]Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States of America

**Also presenting a short oral presentation*

035.* In Situ Click Chemistry Applied to Bunyavirales: From Conventional Drug Design to Enzymes Assembling Their Own Inhibitors like LEGOs®

Laura Garlatti, M.S.[1], Mikael Feracci, Ph.D.[1], Sergio Hernandez, Ph.D.[1], Bruno Canard, Ph.D.[1], Juan Reguera, Ph.D.[1], François Ferron, Ph.D.[1], Karine Alvarez, Ph.D.[1]
[1]*Aix-Marseille Université, Marseille, Bouches-Du-Rhône, France*

052.* Characterization of the N-hydroxypyridinediones (HPD) and the N-hydroxynapthyridinones (HNO) as HBV RNase H inhibitors

Molly Woodson, M.S.[1], Varin Gupta, B.S.[1], Makafui Gasonoo, Ph.D.[2], Sotirios Katsamakas, Ph.D.[3], Marvin Meyers, Ph.D.[2], Grigoris Zoidis, Ph.D.[3], John Tavis, Ph.D.[1]
[1]*Saint Louis University School of Medicine Department of Molecular Microbiology and Immunology, St Louis, United States of America;* [2]*Saint Louis University Department of Chemistry, St Louis, United States of America;* [3]*National and Kapodistrian University of Athens, Department of Pharmacy, Athens, Greece*

053.* Resistance Analysis in a Phase 2 Clinical Trial with the Helicase-Primase Inhibitor Pritelivir in Immunocompromised Adults with Acyclovir Resistant Herpes Simplex Virus (HSV) Infection

Alexander Birkmann, Ph.D.[1], Alexander Greninger, M.D., Ph.D.[2], Meei-Li Huang, Ph.D.[2], Manickam Rangaraju, M.D.[1], Stacy Selke, M.S.[2], Melanie Sumner, M.S.[1], Burkhard Timmler, M.D.[1], Hong Xie, M.S.[2], Haiying Zhu, M.S.[2], Holger Zimmermann, Ph.D.[1], Anna Wald, M.D., MPH[2], Keith Jerome, M.D., Ph.D.[2]
[1]*AiCuris Anti-Infective Cures AG, Wuppertal, Germany;* [2]*Virology Division, Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, United States of America*

100V. Zebrafish Larvae as In Vivo Model for Rift Valley Fever Virus Replication and Pathology

Sebastiaan ter Horst, M.S.[1], Aleksandra Siekierska, Ph.D.[2], Ann-Sofie De Meulemeester, M.S.[2], Arno Cuvry, M.S.[1], Laura Cools, M.S.[1], Peter de Witte, Ph.D.[2], Johan Neyts, Ph.D.[1], **Joana Rocha-Pereira, Ph.D.**[1]
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101. Ex Vivo Midgut Cultures of Aedes Aegypti: A New Tool to Study Infections With Mosquito-Borne Viruses and Antiviral Drugs

Ana Rosales Rosas, M.S.[1], Li-Hsin Li, M.S.[2], Sara Goossens, B.S.[1], Kai Dallmeier, Ph.D.[2], Lanjiao Wang, Ph.D.[1], Leen Delang, Ph.D.[1]
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102V. Designing and Evaluating Neutralizing and Fusion Inhibitory Antiviral Peptides to a Tick-Transmitted Hemorrhagic Fever Virus

Sergio Rodriguez, Ph.D.[1], Dennis Bente, D.V.M., Ph.D.[2], Matteo Porotto, Ph.D.[3]
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103V. Characterizing Humoral Immunity within the Henipahvirus Syrian Hamster Model

Jessica Spengler, D.V.M., Ph.D.[1], **Sergio Rodriguez, Ph.D.**[1]
[1]*CDC, Atlanta, Georgia, United States of America*

**Also presenting a short oral presentation*

104. Remdesivir is Efficacious in a Hamster Model of Yellow Fever Infection and Disease

Justin Julander, Ph.D.[1], Elaine Bunyan, B.S.[2], Robert Jordan, Ph.D.[2], Danielle Porter, Ph.D.[2]
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105V. Heparin Protects Foetal Human Neural Progenitor Cells from ZIKA Virus-Induced Cell Death, While Preserving Their Differentiation Into Mature Neural-Glial Cells In Vitro

Isabel Pagani, Ph.D.[1], Linda Ottoboni, Ph.D.[1], Silvia Ghezzi, M.S.[1], Paola Podini, M.S.[1], Edwin Yates, Ph.D.[2], Elena Brambilla, M.S.[1], Martino Gianvito, M.D., Ph.D.[3], Elisa Vicenzi, Ph.D.[1]
[1]*IRCCS San Raffaele Scientific Institute, Milan, Italy;* [2]*University of Liverpool, Liverpool, United Kingdom of Great Britain and Northern Ireland;* [3]*Vita-Salute San Raffaele University, Milan, Italy*

106V. Discovery of a Chikungunya Virus Entry Inhibitor Targeting Virus Envelope Protein

Leandro Battini, Ph.D. Seeking[1], Daniela Fidalgo, Ph.D.[2], Mariela Bollini, Ph.D.[2], Diego Álvarez, Ph.D.[1]
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107. Gene Expression Profiling Provides Insights Into the Anti-Dengue Mechanism of Metformin

Darren Z. L. Mok, Ph.D. Seeking[1], Clement Yau, Ph.D. Seeking[1], Justin S. G. Ooi, Ph.D.[1], Hwee Cheng Tan, B.S.[1], Summer L. Zhang, B.S.[1], Kuan Rong Chan, Ph.D.[1], Jenny G. H. Low, M.D.[2], Eng Eong Ooi, M.D., Ph.D.[1]
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108. Molecular Mechanisms of Inhibition of Tick-borne Encephalitis Virus by Monoclonal Antibodies

Pavel Svoboda, D.V.M.[1], Jan Haviernik, Ph.D.[1], Jiri Salat, Ph.D.[1], Martin Palus, Ph.D.[2], Pavel Plevka, Ph.D.[3], Tibor Füzik, Ph.D.[3], Marianna Agudelo, M.S.[4], Jennifer Keeffe, Ph.D.[5], Davide Robbiani, M.D., Ph.D.[6], Daniel Ruzek, Ph.D.[2]
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109V. Optimized Design, Synthesis and Biological Evaluation of 2-(4-(phenylsulfonyl) piperazine-1-yl)pyrimidine Analogues as Potent Inhibitors of Chikungunya Virus

Verena Battisti, Ph.D. Seeking[1], Julia Moesslacher, Ph.D.[2], Rana Abdelnabi, Ph.D.[3], Leen Delang, Ph.D.[3], Johan Neyts, Ph.D.[3], Ernst Urban, Ph.D.[1], Thierry Langer, Ph.D.[1]
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110. Pyrimidine Analogs as Potential Antiviral Compounds Against Dengue and Zika Viruses

Agostina Marquez, B.S.[1], Facundo Gallo, B.S.[1], Miguel Peláez, B.S.[2], Mariela Bollini, Ph.D.[1], Cybele García, Ph.D.[1]
[1]*University of Buenos Aires, National Scientific and Technical Research Council, Buenos Aires, Argentina;* [2]*University of Buenos Aires, Buenos Aires, Argentina*

111. Resveratrol and Vitamins: Modulators of Zika Virus Infection

Agostina Marquez, B.S.[1], Priscila Lanza Castronuovo, Ph.D.[2], Mayra Castañeda Cataña, B.S.[1], Claudia Sepúlveda, Ph.D.[1], Mariano Vera, Ph.D.[2], Agustina Alaimo, Ph.D.[1], Cybele García, Ph.D.[1]
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112. The Phenanthroindolizidine (-)-13aR-6-O-desmethyl-antofine Inhibits Zika Virus Replication in Human Cells

Juliano Haddad, Ph.D.[1], Marc Litaudon, Ph.D.[2], Cécile Apel, Ph.D.[2], **Chaker El Kalamouni, Ph.D.**[1]
[1]University of Reunion Island, Saint Denis, France; [2]Institute of Chemistry of Natural substances, Paris, France

113. Porphyrins as Potent Inhibitors of the Entry Process of Tick-Borne Encephalitis Virus

Jiří Ji□í Holoubek, M.S.[1], Luděk Eyer, Ph.D.[2], Marie Vancová, Ph.D.[3], Daniel Rẽzek, Ph.D.[2]
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114. A Yellow Fever Virus NS4B Inhibitor Enhances the Activation of Multiple RNA Sensing Pathways and Induces Pre-mature Death of Infected Cells

Fuxuan Wang, Ph.D.[1], Zhao Gao, Ph.D.[1], Lin Zhang, M.S.[1], Sumangala Darsandhari, Ph.D.[1], Lauren Griffith, M.S.[1], Julia Ma, M.S.[1], Abbie Weight, B.S.[2], Justin Julander, Ph.D.[2], Xinghong Dai, Ph.D.[3], Esther Bullitt, Ph.D.[4], Yanming Du, Ph.D.[1], Ju-Tao Guo, M.D.[1], Jinhong Chang, M.D., Ph.D.[1]
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115. Compound-A Improves Dengue Virus-induced Liver Injury in Immunocompetent Mice via Host Responses

Gopinathan Pillai Sreekanth, Ph.D.[1], Pa-thai Yenchitsomanus, Ph.D.[2], Thawornchai Limjindaporn, M.D., Ph.D.[2]
[1]CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, India; [2]Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

116V. Molecular Basis of Specific Viral RNA Recognition and 5′ End-capping by the Chikungunya Virus nsP1

Dahai Luo, Ph.D.[1], Kuo Zhang, Ph.D.[2], Michelle Law, M.S.[2], Trinh Mai Nguyen, M.S.[2], Yaw Bia Tan, M.S.[2], Melissa Wirawan, Ph.D.[2], Yee-Song Law, Ph.D.[2]
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117V. Targeting CHIKV Replication at the Viral Capping Enzyme nsP1 Through a Combination of Direct Inhibitors and Drugs Interfering With Lipid Metabolism

María-Jesús Pérez-Pérez, Ph.D.[1], Olivier Aïqui-Reboul-Paviet, M.S.[2], Aymeric Neyret, M.S.[2], Ana Lucia Rosales Rosas, M.S.[3], Natalia del Rio, M.S.[1], José-María Orduña, Ph.D.[1], Leen Delang, Ph.D.[3], Laurence Briant, Ph.D.[2]
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118V. A Pre-Membrane Protein PrM D29V Substitution Attenuates a Clinically Tested Live Dengue Vaccine

Milly Choy, Ph.D.[1], Summer Zhang, M.S.[1], Hwee Cheng Tan, B.S.[1], Justin Ooi, Ph.D.[1], Kuan Rong Chan, Ph.D.[1], Eng Eong Ooi, M.D., Ph.D.[1]
[1]Duke-NUS Medical School, Singapore, Singapore

119V. Development of Zika Virus Stable Replicon Cells Expressing Secretory Luciferase

Takayuki Hishiki, Ph.D.[1], Fumihiro Kato, Ph.D.[1], Rieko Suzuki, B.S.[2], Shigeru Tajima, Ph.D.[1], Chang-Kweng Lim, Ph.D.[1], Tomohiko Takasaki, M.D.[2]
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120V. Transcriptional Response to Dengue Virus Infection Reveals Potential Host Targets for Antiviral Intervention

Ilane Hernandez-Morales, D.V.M.[1], Ilse Van Den Wyngaert, Ph.D.[2], Laure Cougnaud, Ph.D.[3], Olivia Goethals, Ph.D.[4], Johan Neyts, Ph.D.[5], Marianne Tuefferd, Ph.D.[2], Jeroen Aerssens, Ph.D.[2], Marnix Van Loock, Ph.D.[4]
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121V. Potent Inhibition of Zika Virus Replication by Favipiravir in HeLa Cells is Linked to Substantial Alterations in Viral Infectivity

Evelyn Franco, Pharm.D.[1], Kaley Hanrahan, M.S.[1], Jieqiang Zhou, B.S.[1], Xun Tao, Ph.D.[1], Eleonora Cella, Ph.D.[2], Taj Azarian, Ph.D.[2], Jurgen Bulitta, Ph.D.[1], Ashley Brown, Ph.D.[1]
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122V. Identification of Druggable Host Factors Crucial for Flavivirus Replication

Min Jie Alvin Tan, Ph.D.[1], Radoslaw M. Sobota, Ph.D.[2], Subhash Vasudevan, Ph.D.[1]
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135. Universal Virucidal Drugs

Francesca Olgiati, M.S.[1], Chiara Medaglia, Ph.D.[2], Pablo Gainza Cirauqui, Ph.D.[1], Bruno Correia, Ph.D.[1], Caroline Tapparel, Ph.D.[2], Francesco Stellacci, Ph.D.[1]
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137. Treatment of a Chronic Human Norovirus Infection of an Immunocompromised Patient With Favipiravir and Nitazoxanide: Assessing Drug Efficacy and the Mechanism of Action Using a Zebrafish Larval Model

Emma Roux, M.S.[1], Juanita Pang, M.S.[2], Alexandra Kreins, M.D., Ph.D.[2], Joseph Standing, Ph.D.[2], Jasper Reymenants, B.S.[1], Johan Neyts, Ph.D.[1], Judith Breuer, M.D., Ph.D.[2], Joana Rocha-Pereira, Ph.D.[1]
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138. Antiviral Activity of 1-O-alkyl-2-O-aryl-sn-glyceryl-3-P-RVn Compounds Against RSV-A2 in Hep-2 and HeLa Cells

Xing-Quan Zhang, Ph.D.[1], Robert Schooley, M.D.[1], James Beadle, Ph.D.[1], Nadjeda Valiaeva, Ph.D.[1], Aaron Carlin, M.D., Ph.D.[1], Joyce Murphy, B.S.[1], **Karl Hostetler, M.D.**[1]
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139. In Vitro and In Vivo Activity of Antimicrobial Peptoids Against Herpes Simplex Virus 1 and 2

Lisa Ryan, Ph.D.[1], Erika Figgins, B.S.[1], Gill Diamond, Ph.D.[1], Natalia Molchanova, Ph.D.[2], Annelise Barron, Ph.D.[2]
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140. Elucidation of the Mechanism of Action of Various Flex-Acyclovir Analogues

Joy Thames, Ph.D. Seeking[1], Emma Lundberg, B.S. Seeking[2], Francesca Esposito, Ph.D.[3], Angela Corona, Ph.D.[3], Enzo Tramontano, Ph.D.[3], Brian Gentry, Ph.D.[2], Katherine Seley-Radtke, Ph.D.[1]
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141. Hits on the Virus RGB Palette

Li-Hsin Li, M.S.[1], Winston Chiu, M.S.[1], Yun-An Huang, Ph.D.[2], Madina Rasulova, Ph.D.[1], Thomas Vercruysse, Ph.D.[1], Hendrik Jan Thibaut, Ph.D.[1], Suzanne Kaptein, Ph.D.[1], Pieter Leyssen, Ph.D.[1], Johan Neyts, Ph.D.[1], Kai Dallmeier, Ph.D.[1]
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142. Broadly Active Antiviral Polyphenolic Compounds Inhibit SAR-CoV-2 in Culture

Chloe Monet-Murrell, B.S.[1], Consuelo Correa-Sierra, M.D., Ph.D.[1], Seyedeh Hosseini, Ph.D.[2], Devon Schartz, B.S.[2], James Connelly, B.S.[2], Shaohui Yu, B.S.[2], Jody Cameron, M.S.[1], Frederick West, Ph.D.[2], Luis Schang, D.V.M., Ph.D.[1]
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143. PRTX007, a TLR7 Agonist, Demonstrates Broad-Spectrum Antiviral Activity and is Appropriate for Pandemic Preparedness

James Appleman, Ph.D.[1], Stephen Webber, Ph.D.[1], Scott Zook, M.S.[1], Curtis Scribner, M.D.[2], Richard Daniels, B.S.[1]
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144. Mining Chemical Bioactivity Data to Identify Broad-Spectrum Antiviral Agents

Holli-Joi Sullivan, M.S.[1], Cleber Melo-Filho, Ph.D.[2], Richard Eastman, Ph.D.[3], Alexey Zakharov, Ph.D.[3], Eugene Muratov, Ph.D.[2], Alexander Tropsha, Ph.D.[2]
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145V. Ribavirin Enhances the Anti-Coronavirus Activity of Molnupiravir and GS-441524 in Ex Vivo Model

Thuc Nguyen Dan Do, M.S.[1], Bernadett Boda, Ph.D.[2], Samuel Constant, Ph.D.[2], Dirk Jochmans, Ph.D.[1], Johan Neyts, Ph.D.[1]

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146. Development of a High-Throughput Screening Pipeline for Potential Inhibitors of SARS-Cov-2 Replication Using the Caps-It System, an Automated Lab-in-a-Box

Winston Chiu, Ph.D. Seeking[1], Dirk Jochmans, Ph.D.[1], Laura Vangeel, Ph.D.[1], Steven De Jonghe, Ph.D.[1], Kayvan Jahed, M.S.[1], Joost Schepers, M.S.[1], Thibault Francken, B.S.[1], Emmanuel André, M.D., Ph.D.[1], Johan Neyts, Ph.D.[1], Pieter Leyssen, Ph.D.[1]

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147V. Design and Synthesis of Flex AT-527 as a Potential Antiviral Therapeutic

Tyler Carlyle, B.S. Seeking[1], Charles Waters, Ph.D. Seeking[1], Carmine Varrichio, Ph.D.[2], Andrea Brancale, Ph.D.[2], Katherine Seley-Radtke, Ph.D.[1]

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148. Single Infectious Unit Sequencing Revealed That Alphaviruses Maintain Their Infectious Populations Within a Narrow Genetic Heterogeneity Range

Brian Alejandro, M.S.[1], Eunjung Kim, B.S.[1], **Donghoon Chung, Ph.D.**[1]

[1]University of Louisville, Louisville, Kentucky, United States of America

149. Synthesis and Biological Evaluation of a Flexible Nucleoside Analogue of AT-527

Charles Waters, Ph.D. Seeking[1], Evan Carlyle, B.S. Seeking[1], Shuaishuai Liu, M.S.[1], Apurv Rege, M.S.[1], Consuelo Correa-Sierra, Ph.D.[2], Luis Schang, D.V.M., Ph.D.[2], Charles Bieberich, Ph.D.[1], Katherine Seley-Radtke, Ph.D.[1]

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150. Design, Synthesis, and Enzymatic Activation of the Nucleobase Analogue T-1105

Olivia Kannas, B.S.[1], Johanna Huchting, Ph.D.[2], Brian Gentry, Ph.D.[1], Chris Meier, Ph.D.[2]

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151. Broad-spectrum Inhibitors Against the SARS CoV-2 NSP14 and Flavivirus Methyltransferases

Qamar Bashir, Ph.D.[1], Subodh Samrat, Ph.D.[1], Xiangmeng Wu, Ph.D.[1], Yiding Huang, M.S.[1], Zhong Li, M.S.[1], Qing-Yu Zhang, Ph.D.[1], **Hongmin Li, Ph.D.**[1]

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152V. Design, Synthesis and Biological Evaluation of LAVR-289, a New Broad-spectrum Antiviral ANP

Tuniyazi Abuduaini, Ph.D.[1], Kathleen Solmont, Ph.D.[1], Thomas Mathieu, M.S.[1], Patrick Favetta, Ph.D.[1], Maxime Bessieres, Ph.D.[1], Graciela Andrei, Ph.D.[2], Robert Snoeck, Ph.D.[2], Franck Gallardo, Ph.D.[3], **Luigi Agrofoglio, Ph.D.**[1]

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153V. Synthesis of Cyclic Dinucleotides as Modulators of STING, a Pivotal Protein in Immunity and Antiviral Diseases

Jérémy Magand, M.S.[1], Andrea Ojeda-Porras, Ph.D.[1], Stéphanie Rose, M.S.[2], Valérie Quesniaux, Ph.D.[2], Vincent Roy, Ph.D.[1], **Luigi Agrofoglio, Ph.D.**[1]
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154V. A Large-scale Drug Repositioning Survey Identifies Clofazimine that Broadly Inhibits Coronaviruses Including SARS-CoV-2

Shuofeng Yuan, D.V.M., Ph.D.[1], Jasper Fuk-Woo Chan, M.D.[1], Kwok-Yung Yuen, M.D.[1]
[1]The University of Hong Kong, Hong Kong

155V. Viral Two-Metal Ion Dependent Enzymes as Antiviral Targets

Savithri Weerasooriya, Ph.D.[1], Andrea Pruijssers, Ph.D.[2], Laura Stevens, Ph.D.[2], Dung Do, Ph.D.[3], Lee Wright, Ph.D.[3], Mark Denison, M.D.[2], Dennis Wright, Ph.D.[3], **Sandra Weller, Ph.D.**[1]
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156. AI-Derived Antibodies are Novel, Diverse and Pharmacologically Active Against Multiple SARS-CoV-2 Strains

Cristina Moldovan Loomis, Ph.D.[1], Megan Sprague, B.S.[1], Andrew Asakawa, B.S.[1], Kathryn McLean, B.S.[1], Lindsay Pautsch, B.S.[1], Gregory Neveu, Ph.D.[2], Valentin Simioni, B.S.[2], Laurence Somody, Ph.D.[2], Antoine Alam, Ph.D.[2], Randal Ketchem, Ph.D.[1], Rutilio Clark, Ph.D.[1]
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157V. Oral Heat Shock Protein 90 (Hsp90) Inhibitor SNX-5422 Attenuates SARS-CoV-2 Replication And Dampens Inflammation In Airway Cells

Ria Goswami, Ph.D.[1], Veronica Russell, B.S.[2], Joshua Tu, B.S.[2], Charlene Thomas, M.S.[1], Philip Hughes, Ph.D.[2], Francine Kelly, B.S.[2], Stephanie Langel, Ph.D.[2], Justin Steppe, B.S.[2], Scott Palmer, M.D.[2], Timothy Haystead, Ph.D.[2], Maria Blasi, Ph.D.[2], Sallie Permar, M.D., Ph.D.[1]
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158. Oral Pharmacokinetics and Antiviral Activity of 1-O-alkyl-2-O-aryl-sn-glyceryl-3-P-RVn Compounds Predicts Broad Spectrum Efficacy against RNA Viruses

Aaron Carlin, M.D., Ph.D.[1], James Beadle, Ph.D.[1], Nadejda Valiaeva, Ph.D.[1], Rachel McMillan, Ph.D. Seeking[1], Alex Clark, Ph.D.[1], William Bray, B.S.[1], Aaron Garretson, B.S.[1], Xing-Quan Zhang, M.D.[1], Joyce Murphy, B.S.[1], Michael Lo, Ph.D.[2], Robert Schooley, M.D.[1], **Karl Hostetler, M.D.**[1]
[1]University of California, San Diego, La Jolla, California, United States of America; [2]US Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

159V. General Lipoperoxidators Do Not Target Virion Lipids Specifically

Consuelo Correa-Sierra, M.D., Ph.D.[1], Luis Schang, D.V.M., Ph.D.[1]
[1]Cornell University, Ithaca, New York, United States of America

160V. Characterization of the Susceptibility of Specific SARS-CoV-2 nsp12 Substitutions to Remdesivir Using a Coronavirus Reverse Genetic System

Venice Du Pont, Ph.D.[1], Laura Stevens, M.S.[2], Andrea Pruijssers, Ph.D.[2], Amelia George, M.S.[2], Tia Hughes, M.S.[2], Xuping Xie, Ph.D.[3], Pei-Yong Shi, Ph.D.[3], Danielle Porter, Ph.D.[1], Tomas Cihlar, Ph.D.[1], Mark Denison, M.D.[2], John Bilello, Ph.D.[1]

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161V. Adaptation of Heartland Virus in AG129 Mice Leads to Severe Disease that can be Effectively Treated with the Ribonucleoside Analog, EIDD-2749

Jonna Westover, Ph.D.[1], Gregory Bluemling, Ph.D.[2], Philip Hicks, B.S.[3], Gabriellle Rock, B.S.[1], Kirsten Boardman, B.S.[1], Manu Saindane, Ph.D.[2], Alexander Kolykhalov, Ph.D.[2], Paul Bates, Ph.D.[4], George Painter, Ph.D.[2], Brian Gowen, Ph.D.[1]

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162V. Development of N-Substituted Furopyrroles as EBOV and MARV Anti-filoviral Glycoprotein Inhibitors.

Destiny Durante, Ph.D. Seeking[1], Irina Gaisina, Ph.D.[1], Laura Cooper, Ph.D.[1], Ryan Bott, B.S.[1], RuthMabel Boytz[4], Norton Peet, Ph.D.[2], Robert Davey, Ph.D.[3], Terry Moore, Ph.D.[1], Lijun Rong, Ph.D.[1]

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163V. Exploring the Morphology of the Host Cell to Open New Possibilities in Drug Repurposing

Marianna Tampere, Ph.D.[1], Jonne Rietdijk, M.S.[2], Hanna Axelsson, M.S.[1], Jayasankar Kaimal, Ph.D.[3], Swapnil Potdar, M.S.[4], Philipp Ianevski, M.S.[4], Duncan Njenda, Ph.D.[5], Maris Lapins, Ph.D.[2], Trang Le, M.S.[6], Ulrika Axelsson, Ph.D.[3], Anna Bäckström, M.S.[7], Elisabeth Moussaud-Lamodière, Ph.D.[8], Jani Saarela, Ph.D.[4], Brinton Seashore-Ludlow, Ph.D.[8], Polina Georgiev, Ph.D.[2], Emma Lundberg, Ph.D.[3], Ola Spjuth, Ph.D.[2], Jordi Carreras-Puigvert, Ph.D.[2], Charlotte Stadler, Ph.D.[7], Päivi Östling, Ph.D.[8]

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164V. Integrated Pipeline Using ANCHOR™ Tagged Autofluorescent Viruses for Accelerated Discovery of Antiviral Molecules

Sandrine Kappler-Gratias, Ph.D.[1], Thomas Figueroa, Ph.D.[1], Charlotte Quentin-Froignant, Ph.D.[1], Elie Marcheteau, Ph.D.[1], Sokunthea Top, Ph.D.[1], Franck Gallardo, Ph.D.[1]

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165. Combinations of Directly Acting and Host-targeting Antiviral Drugs Confer Synergistic Suppression of SARS-CoV-2 Infection

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167. Broad-Spectrum In Vitro Antiviral Activity of ODBG-P-RVn: An Orally-Available, Lipid-Modified Monophosphate Prodrug of Remdesivir Parent Nucleoside (GS-441524)

Michael Lo, Ph.D.[1], Punya Shrivastava-Ranjan, Ph.D.[1], Payel Chatterjee, M.S.[1], Mike Flint, Ph.D.[1], James Beadle, Ph.D.[2], Nadejda Valiaeva, Ph.D.[2], Joyce Murphy, M.S.[2], Robert Schooley, M.D.[2], Karl Hostetler, Ph.D.[2], Joel Montgomery, Ph.D.[1], Christina Spiropoulou, Ph.D.[1]

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185. Prophylactic Efficacy of Intranasal Administration of IgY Antibodies Targeting Receptor Binding Domain Against SARS-Cov-2 Infection in a Transgenic Mouse Model

Ayman Abbas, Ph.D.[1], Sherif El-Kafrawy, Ph.D.[1], Abby Odle, M.S.[2], Ahmed Hassan, Ph.D.[1], Stanley Perlman, M.D., Ph.D.[2], Alimuddin Zumla, Ph.D.[3], Esam Azhar, Ph.D.[1]

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186. 1-O-Alkyl-2-O-substituted-sn-glyceryl Esters of GS-441524 5′-monophosphate: Synthesis and Antiviral Activity against SARS-CoV-2

James Beadle, Ph.D.[1], Aaron Carlin, M.D.[1], Nadejda Valiaeva, Ph.D.[1], Alex Clark, Ph.D.[1], William Bray, Ph.D.[1], Aaron Garretson, Ph.D.[1], Xing-Quan Zhang, Ph.D.[1], Joyce Murphy, B.S.[1], Robert Schooley, M.D.[1], Karl Hostetler, M.D.[1]

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188. A Structural Model of the SARS-CoV-2 Replication and Transcription Complex

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189. In Silico Discovery of Novel Potential RNA-binding Small Molecules Against SARS-CoV-2

Carmine Varricchio, Ph.D.[1], Gregory Mathez, M.S.[2], Andrea Brancale, Ph.D.[1], Valeria Cagno, Ph.D.[2]

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190. Natural-derived Inhibitors of SARS-CoV-2 nsp13 Unwinding and ATPase Activities a Starting Scaffold to Develop Antiviral Agents

Angela Corona, Ph.D.[1], Krzysztof Wycisk, Ph.D.[2], Carmine Talarico, Ph.D.[3], Candida Manelfi, M.S.[3], Jessica Milia, M.S.[1], Rolando Cannalire, Ph.D.[4], Francesca Esposito, Ph.D.[1], Philip Gribbon, Ph.D.[5], Andrea Zaliani, Ph.D.[5], Daniela Iaconis, Ph.D.[3], Andrea Beccari, Ph.D.[3], Vincenzo Summa, Ph.D.[4], Marcin Nowotny, Ph.D.[2], Enzo Tramontano, Ph.D.[1]

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191. Development of a Rapid MoA-based Potency Bioassay using Virus-like Particles for Assessment of SARS-CoV-2 Entry Inhibitors

Jonathan Mitchell, Ph.D.[1], Jamison Grailer, Ph.D.[1], Jim Hartnett, M.S.[1], Denise Garvin, M.S.[1], Frank Fan, Ph.D.[1], Mei Cong, Ph.D.[1], Zhi-Jie Jey Cheng, Ph.D.[1]
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192V. Comparative Infectivity of Emerging SARS-CoV-2 Variants in Human Airway Epithelial Cell Cultures

Thuc Nguyen Dan Do, M.S.[1], Sandra Claes, B.S.[1], Dominique Schols, Ph.D.[1], Dirk Jochmans, Ph.D.[1], Johan Neyts, Ph.D.[1]
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193. A New Mouse Model of SARS-Cov-2 Infection as a Tool for Evaluating COVID-19 Vaccines and Therapeutics

Daniel Ruzek, Ph.D.[1], Jan Prochazka, Ph.D.[2], Martin Palus, Ph.D.[1], Vaclav Honig, Ph.D.[1], Radislav Sedlacek, Ph.D.[2], Davide Robbiani, M.D., Ph.D.[3], Luca Varani, Ph.D.[3]
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194. Cyclophilin Inhibitors Enhance Cell-Autonomous Antiviral Immunity to Inhibit Coronavirus Infection

John Mamatis, B.S.[1], Che Colpitts, Ph.D.[1]
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195. Disruption of Conserved Heparan Sulfate-Dependent Attachment as an Antiviral Strategy for Emerging Coronaviruses

Emmanuelle LeBlanc, M.S.[1], Kimberley Siwak, B.S. Seeking[1], Youjin Kim, B.S.[1], Daniel Whalen, B.S.[1], Chantelle Capicciotti, Ph.D.[1], Che Colpitts, Ph.D.[1]
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196. Discovery of Highly Potent Oligonucleotides Targeting SARS-CoV-2

Ruchika Jaisinghani, M.S.[1], Suping Ren[1], Aneerban Bhattacharya[1], Saul Martinez Montero[1], John Cortez[1], Jacquelyn Sousa[1], Dana Cho[1], Antitsa Stoycheva[1], Sandra Chang[1], Vikrant Gohil[1], Pei-Yong Shi[2], Hongjie Xia[2], Julian Symons[1], David Smith[1], Lawrence Blatt[1], Leonid Beigelman[1], Jin Hong[1], Andreas Jekle[1]
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197. An Ultrapotent DARPin Molecule as a Broadly Neutralizing Inhaled Therapeutic Against COVID

Zhilei Chen, Ph.D.[1]
[1]Texas A&M University Health Science Center, College Station, Texas, United States of America

198. The Pathogenesis of COVID-19 Depends on SARS-Cov-2 Exposure Dose and Frequency and Persists After Recovery from Clinical Infection in a Mouse Model

Luis Schang, D.V.M., Ph.D.[1], Rodrigo Santos, Ph.D.[1], Chloe Monet-Murrell, B.S.[1], Julius Judd, B.S.[1], Natalia Komarova, Ph.D.[2], Dominik Wodarz, Ph.D.[2], Elena Demeter, D.V.M., Ph.D.[1], Nicole Kushner, B.S.[1], Maureen Fernandes, D.V.M., Ph.D.[1], Leandro Cardia Caserta, D.V.M., Ph.D.[1], Diego Diel, D.V.M., Ph.D.[1], Cédric Feschotte, Ph.D.[1], John Lis, Ph.D.[1]

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199. Replication and Antiviral Activity of MERS and SARS-Cov-2 Variants in a Highly Specialized 3D Mucociliary Tissue Model Consisting of Normal, Human-Derived Tracheal/Bronchial Epithelial Cells

Kie Hoon Jung, Ph.D.[1], Mark Summers, B.S.[1], James Hansen, B.S.[1], Jung-Ae Choi, M.S.[1], Jonna Westover, Ph.D.[1], Brett Hurst, Ph.D.[1]

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200. Structural Basis of Nucleotide Recognition by the SARS-CoV-2 RNA Dependent RNA Polymerase

Brandon Malone, Ph.D. Seeking[1], Jason Perry, Ph.D.[2], James Chen, Ph.D.[3], Paul Dominic Olinares, Ph.D.[1], Todd Appelby, Ph.D.[2], Joy Feng, Ph.D.[2], John Bilello, Ph.D.[2], Elizabeth Campbell, Ph.D.[1], Seth Darst, Ph.D.[1]

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201. A Hypothesis: Covid-induced Micro-clots are Formed Only During Sero-conversion.

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202V. Remdesivir Retains Potent Antiviral Activity Against the SARS-CoV-2 Delta Variant and Other Emergent Variants

Xianghan Lu, M.D.[1], Jared Pitts, Ph.D.[1], Venice Du Pont, Ph.D.[1], Nicholas Riola, B.S.[1], Tomas Cihlar, Ph.D.[1], Danielle Porter, Ph.D.[1], John Bilello, Ph.D.[1]

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203V. Synthetic Host Defense Peptide Inhibits SARS-CoV-2 Replication In Vitro

Rhodri Harfoot, Ph.D.[1], Francesca Hills, B.S.[1], Leonor Hernandez, M.D.[1], Blair Lawley, Ph.D.[1], Joanna Kuang, M.S.[1], Tom Bird, Ph.D.[2], Davide Comoletti, Ph.D.[2], Evan Haney, Ph.D.[3], Reza Falsafi, Ph.D.[3], Robert Hancock, Ph.D.[3], Daniel Pletzer, Ph.D.[1], **Miguel Quiñones-Mateu, Ph.D.**[1]

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207V. Immune and Inflammatory Profile in Critically Ill COVID-19 Patients After Recovery

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208V. Immunomodulatory Effect on Dendritic Cells and T-cells of α-1-thymosine (α1Thy) in Response to SARS-CoV2

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209V. SARS-CoV-2 Omicron and Pan-Variant Neutralization Activity of Ensovibep: a DARPin Therapeutic Candidate for Treatment of Covid-19

Sylvia Rothenberger, Ph.D.[1], Marcel Walser, Ph.D.[2], Francesca Malvezzi, Ph.D.[2], Daniel Hurdiss, Ph.D.[3], Jennifer Mayor, Ph.D.[1], Hector Moreno, Ph.D.[1], Sarah Ryter, B.S.[4], Nicole Liechti, Ph.D.[4], Andreas Bosshart, Ph.D.[2], Susanne Mangold, M.S.[2], Filip Radom, Ph.D.[2], Charles Knutson, Ph.D.[5], Krishnan Ramanathan, Ph.D.[6], Olivier Engler, Ph.D.[4], **Michael Stumpp, Ph.D.**[2]
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210V. An Interaction of Early Pregnancy-associated Protein-1 with SARS Cov-2 S Protein: Implications in Vertical Transmission of SARS-2 CoV-2

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211V. Identification of Potent Inhibitors of SARS-CoV-2 Exoribonuclease by Fluorescence Polarization Assay

Sergio Hernandez, Ph.D.[1], Carolina Trajano, B.S.[1], Priscila El Kazzi, M.S.[1], Mikael Feracci, Ph.D.[1], Clemence Mondielli, Ph.D.[2], Laura Garlatti, M.S.[1], Rafik Kaci, M.S.[1], Philippe Cotelle, Ph.D.[3], Franck Touret, Ph.D.[4], Bruno Coutard, Ph.D.[4], Xavier De Lamballerie, Ph.D.[4], Etienne Decroly, Ph.D.[1], François Ferron, Ph.D.[1], Karine Alvarez, Ph.D.[1], Bruno Canard, Ph.D.[1]
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212V. PRO-2000 Inhibits SARS-CoV-2 Replication by Interfering with Spike-heparin Binding

Evelien Vanderlinden, Ph.D.[1], Arnaud Boonen, M.S.[1], Sam Noppen, M.S.[1], Piet Maes, Ph.D.[1], Robert Snoeck, M.D., Ph.D.[1], Annelies Stevaert, Ph.D.[1], Lieve Naesens, Ph.D.[1], Dominique Schols, Ph.D.[1], Graciela Andrei, Ph.D.[1]
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214V. Mutations L50F, E166A and L167F in SARS-CoV-2 3CLpro are Selected by a Protease Inhibitor in vitro and are Associated with Resistance

Cheng Liu, Ph.D.[1], Kim Donckers, Ph.D.[2], Sarah K. Stevens, M.S. [1], Steven De Jonghe, Ph.D. [2], Antitsa Stoycheva, Ph.D. [1], Dorothée Bardiot, Ph.D.[3], Sandro Boland, Ph.D. [3], Lawrence M. Blatt, Ph.D. [1], Leonid Beigelman, Ph.D. [1], Julian A. Symons, Ph.D.[1], Piet Maes, Ph.D.[2], Bert Vanmechelen, Ph.D. [2], Pierre Raboisson, Ph.D.[4], Patrick Chaltin, Ph.D.[5], Arnaud Marchand, Ph.D. [3], Koen Vandyck, Ph.D.[4], Jerome Deval, Ph.D. [1], Johan Neyts, Ph.D. [2], Dirk Jochmans, Ph.D. [2]
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215V. Novel Antiviral Activity of PAD Inhibitors Against Human Beta-coronaviruses HCoV-OC43 and SARS-CoV-2

Selina Pasquero, Ph.D.[1], Francesca Gugliesi, Ph.D. [1], Gloria Griffante, Ph.D. [1], Valentina Dell'Oste, Ph.D.[1], Matteo Biolatti, Ph.D. [1], Camilla Albano, M.S. [1], Greta Bajetto, M.S. [1], Serena Delbue, Ph.D.[2], Lucia Signorini, Ph.D. [2], Maria Dolci, Ph.D. [2], Marco De Andrea, M.D., Ph.D. [1]
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230. Synthesis and Antiviral Evaluation of β-L-[5-(E-2-bromovinyl)-2-(hydroxymethyl)-1, 3-(dioxolane-4-yl) uracil (L-BHDU) Prodrugs Against Varicella Zoster Virus (VZV)

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231V. Pre-clinical Evaluation of LAVR-289, a Novel Potent CMV Replication Inhibitor

Tuniyazi Abuduaini, Ph.D.[1], Chloé Jacquet, Ph.D.[2], Perrine Coste-Mazeau, M.D., Ph.D.[2], Joy Ijil-Musanga, B.S.[2], Sébastien Hantz, Ph.D.[2], Vincent Roy, Ph.D.[1], Sophie Alain, M.D., Ph.D.[2], **Luigi Agrofoglio, Ph.D.**[1]
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232. Antiviral Activity of Peptide A-3302-B Isolated From a Marine Bacterium Micromonospora sp. Against Herpes Simplex Virus Type 2.

Irene Arduino, M.S.[1], Massimo Rittà, M.D., Ph.D.[1], Rachele Francese, Ph.D.[1], Stefania Raimondo, Ph.D.[1], Sanya Sureram, M.S.[2], Prasat Kittakoop, Ph.D.[2], David Lembo, Ph.D.[1], Manuela Donalisio, Ph.D.[1]
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233. Optimization and Structure-Activity Relationship Studies of 1-Hydroxy-1, 8-naphthyridin-2(1H)-one-based Derivatives as Potent Inhibitors for Hepatitis B Virus Replication

Makafui Gasonoo, Ph.D.[1], Molly Woodson, M.S.[1], Qilan Li, Ph.D.[1], Maryam Zangi, M.S.[1], John Tavis, Ph.D.[1], Marvin Meyers, Ph.D.[1]
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234. α-Hydroxytropolone HBV RNase H Inhibitors: Effects of Troponoids on Mitochondrial Function and Cytotoxicity

Daniel Bradley, B.S.[1], Austin O'Dea, M.S.[1], Molly Woodson, M.S.[1], Qilan Li, Ph.D.[1], Nathan Ponzar, B.S.[1], Alaina Knier, B.S.[1], Bruce Rogers, Ph.D.[2], Ryan Murelli, Ph.D.[3], John Tavis, Ph.D.[1]
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235. Chimeric Capsid Inhibitors Carrying Proteolysis Targeting Moieties Induce HBV Capsid and Core Protein Degradation

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236. Mechanism and Regulation of Intracellular Amplification of Hepatitis B Virus Covalently Closed Circular DNA and Implications in Antiviral Drug Development

Qiong Zhao, M.D.[1], Liudi Tang, Ph.D.[1], Jin Hu, Ph.D.[1], Yuhuan Li, Ph.D.[2], Tianlun Zhou, Ph.D.[1], Jinhong Chang, Ph.D.[1], Ju-tao Guo, M.D.[1]
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237. The Design and Synthesis of Reverse Fleximers of L-Nucleoside Analogues

Christianna Kutz, Ph.D. Seeking[1], Antonio Coluccia, Ph.D.[2], Marianna Bufano, Ph.D. Seeking[2], Grayson Pipher, B.S. Seeking[1], Owen Sparr, B.S. Seeking[1], Romano Silvestri, Ph.D.[2], Katherine Seley-Radtke, Ph.D.[1]
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238. Novel Hepatoselective Dihydroquinolizinones for HBV Surface Antigen (HBsAg) Reduction

Nicky Hwang, B.S.[1], Liren Sun, M.S.[1], Daisy Noe, B.S.[1], Tianlun Zhou, Ph.D.[1], Timothy Block, Ph.D.[1], **Yanming Du, Ph.D.**[1]
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239. Predicted Structure of the Hepatitis B Virus Polymerase Reveals an Ancient Conserved Protein Fold

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240V. L-BHDU Prodrugs are Highly Effective Against Varicella Zoster Virus in vivo

Megan Lloyd, Ph.D.[1], Dongmei Liu, M.S.[1], Uma Singh, Ph.D.[2], Ananda Konreddy, Ph.D.[2], Yugandhar Kothapalli, Ph.D.[2], Chung Chu, Ph.D.[2], Jennifer Moffat, Ph.D.[1]
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246V. Differential Inhibition of PAPD5 and PAPD7 by Small-Molecule HBV RNA Destabilizers from Dihydroquinolizinone and Tetrahydropyrido[4,3-d]pyrimidine Chemical Series

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260. Antiviral Activity of the Clinical Stage TLR7 Agonist Prodrug PRTX007 in a Murine Respiratory Syncytial Virus Animal Model

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261. Unravelling the Anti-Influenza Effect of Flavonoids: Experimental Validation of Luteolin and its Congeners as Potent Influenza Endonuclease Inhibitors

Katerina Radilova, M.S.[1], Robert Reiberger, M.S.[1], Michal Kral, M.S.[1], Vaclav Zima, Ph.D.[1], Pavel Majer, Ph.D.[1], Jiri Brynda, Ph.D.[1], Jan Konvalinka, Ph.D.[1], Ales Machara, Ph.D.[1], Milan Kozisek, Ph.D.[1]

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262V. Monitoring Susceptibility of Seasonal Influenza A and B Viruses to Baloxavir in the United States, 2018-2021

Mira Patel, Ph.D.[1], Ha Nguyen, Ph.D.[2], Anton Chesnokov, M.S.[1], Daniel Flanigan, M.S.[2], Vasiliy Mishin, Ph.D.[1], David Wentworth, Ph.D.[1], Larisa Gubareva, M.D., Ph.D.[1]

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263V. Characterization of Influenza B Viruses with Reduced Susceptibility to Influenza Neuraminidase Inhibitors.

Sook Kwan Brown, M.S.[1], Yeu-Yang Tseng, Ph.D.[1], Ammar Aziz, Ph.D.[1], Mariana Baz, Ph.D.[1], Ian Barr, Ph.D.[1]

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265. Comparison of Four Enterovirus D68 Clinical Isolates from 2018 for Neurovirulence in Type I Interferon Receptor (IFNAR) Knock-out Mice

Scott Gibson, B.S.[1], Brett Hurst, Ph.D.[1], E. Bart Tarbet, Ph.D.[1], Zoe Meyer, B.S.[1]

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280V. Tissue Replication and Mucosal Swab Detection of Sosuga Virus in Syrian Hamsters in the Absence of Clinical Disease

Jessica Spengler, D.V.M., Ph.D.[1], Stephen Welch, Ph.D.[1], Sarah Genzer, D.V.M.[1], Teresa Sorvillo, Ph.D.[1], Jessica Harmon, M.S.[1], JoAnn Coleman-McCray, B.S. Seeking[1], Florine Scholte, Ph.D.[1], Shilpi Jain, Ph.D.[1], Punya Shrivastava-Ranjan, Ph.D.[1], Amy Schuh, Ph.D.[1], Jonathan Towner, Ph.D.[1], Joel Montgomery, Ph.D.[1], César Albariño, Ph.D.[1], Christina Spiropoulou, Ph.D.[1]

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281. Synthesis and Evaluation of Hydroxymethyl-propyl-phosphonates-diphosphates (HPMPG/T)

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282V. Optimization of a Miniaturized, Cell-based, Screening Assay to Discover Rabies Virus Antivirals

Xinyu Wang, M.S.[1], Winston Chiu, M.S.[1], Ashley Banyard, Ph.D.[2], Anthony Fooks, Ph.D.[2], Johan Neyts, Ph.D.[1], Dirk Jochmans, Ph.D.[1]

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283V. Discovery and Mechanistic Investigations of Phenylalanine Derivatives as Novel HIV-1 Capsid Inhibitors

Shujing Xu, Ph.D.[1], Xujie Zhang, M.S.[1], Lin Sun, Ph.D.[1], Dang Ding, M.S.[1], Simon Cocklin, Ph.D.[2], Christophe Pannecouque, Ph.D.[3], Xinyong Liu, Ph.D.[1], Peng Zhan, Ph.D.[1]

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287. Activity of Islatravir Against Diverse Primary Isolates of HIV-1 and Putative Resistant and Hypersensitive Mutants

Maria E. Cilento, M.S.[1], Philip R. Tedbury, Ph.D. [1], Obiaara B. Ukah, Ph.D.[2], Eiichi N. Kodama, Ph.D.[3], Hiroaki Mitsuya, Ph.D.[4], Michael A. Parniak, Ph.D.[5], Karen A. Kirby, Ph.D. [1], Stefan G. Sarafianos, Ph.D. [1]

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001. What Role Can Foundations Play in Driving Innovation Globally in Antiviral Development?

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The COVID-19 pandemic highlighted the need to be better prepared in the future to deal with an emerging respiratory virus. We cannot rely on repurposing. Having access to most registered drugs and drugs in development did not produce orally-available antivirals active against SARS-CoV-2 (although there was some success with disease-modifying agents). Antiviral drugs with the appropriate spectrum of activity can protect against a new pandemic threat until a vaccine can be developed and deployed. They are likely to be the cheapest and most scalable product type in the arsenal, being easily manufactured, stockpiled, and distributed. Drug candidates should be suitable for post-exposure prophylaxis or treatment of mild disease. They should possess oral activity, a good safety profile, low cost of goods, be easy to deliver and suitable for combination. Equitable access to resulting products must be ensured. The global research community must be engaged. Foundations can ensure global reach by linking funding to actionable global access provisions. At the Bill & Melinda Gates Foundation we have chosen to focus on viral families of concern including Coronaviruses (SARS-1, SARS-2, MERS) Orthomyxoviruses (Influenza A) and Paramyxoviruses (Nipah, Hendra). In addition, we should be prepared for "Disease X", caused by a virus that is not amongst those that have been studied. The foundation is coordinating its funding with other organizations to create a jointly managed portfolio of projects that will contribute drug candidates to the global antiviral armamentarium.

002. Drug Development for Viruses of Pandemic Potential, Where Are We Now and Where Are We Going?

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A critical element of the response to the global SARS-CoV-2 pandemic has been the development of therapeutics to treat COVID. Therapeutics for hospitalized patients and neutralizing monoclonal antibodies for outpatient treatment have reduced COVID morbidity and mortality. Looking forward we have added potent, oral medications that can easily be delivered to prevent hospitalization and death. The effective delivery of effective vaccines that prevent acquisition and disease, combined with antivirals that could be used as pre-exposure prophylaxis, post-exposure prophylaxis and out-patient treatment to prevent progression to hospitalization and death is the ideal strategy to help defeat this and future pandemics. The development of safe, potent, and simple to deliver antivirals for SARS-CoV-2 is based upon the strategies used to develop medications for other pandemic viruses-HIV and HCV and influenza. For pandemic response against SARS-CoV-2 and related Coronaviruses, drug development is focusing on strategies to maintain potency and breath. When we shift our focus from pandemic response to pandemic preparedness, there are key principles that need to be applied as we collectively seek to develop antivirals, vaccines and monoclonal antibodies against viruses from the targeted seven viral families. By creating platforms for the rapid production of interventions, vaccines, monoclonal antibodies, and therapeutics, we can be prepared for outbreaks in the future and quickly deploy these interventions. To achieve this vision there needs to be global cooperation to implement and maintain a robust pandemic preparedness plan.

003. Developing An Oral Antiviral Agent in a Pandemic: The Evolution of Molnupiravir (EIDD-2801, MK-4482) as a Treatment for COVID-19

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In Q4 2019 the ribonucleoside analog molnupiravir was in late pre-clinical development for treatment of seasonal influenza with a secondary indication for treatment of highly pathogenic coronavirus infection. In January of 2020 in response to mounting concern over the emergence of a novel, highly virulent coronavirus, the focus was shifted to treatment of coronavirus infections, and development was accelerated. Despite the development of vaccines and

monoclonal antibodies, antiviral drugs with potent activity against SARS-CoV-2, the causative agent of COVID-19, would also be needed to address the pandemic. Experience with other respiratory RNA viruses suggested there would be an increasing need for drugs to cover vaccine failure including from emerging SARS-CoV-2 variants against which existing vaccines and monoclonal antibodies might be less effective. Millions of people are immune-compromised and unable to mount a fully protective immune response after vaccination. Here, we describe the development of molnupiravir, a broad-spectrum, orally bioavailable antiviral agent originally designed for treatment of encephalitic New World alphavirus infections, into a drug for the treatment of COVID-19. Molnupiravir is quickly metabolized to N^4-hydroxycytidine-5′-triphophate, which acts as a competitive alternative substrate for the virally encoded RNA-dependent RNA-polymerase. Owing to its ability to tautomerize, incorporation of N^4-hydroxycytidine-5′-monophosphate into nascent chain RNA results in an increase in transition mutations and an extremely rapid loss of viral replication competence and virulence. Efficacy has been demonstrated in clinical studies, and emergency use authorization for treatment of certain patients with mild to moderate COVID-19 has been granted for molnupiravir in multiple countries.

004. Structure-guided Antiviral Drug Discovery – A Tale of Two Viruses

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Respiratory viruses can present the most challenging and life-threatening infections to humans and the current COVID-19 pandemic is testament to that potential. Influenza viruses through to coronaviruses continue to provide humanity with great concern due to the rapid onset of disease, as well as their potential overwhelming direct life-threatening impact on lung function and other organ systems. Furthermore, the rapid development of mutants may also lead to further complications in the employment of existing vaccines, where they exist. Consequently, antiviral drug discovery strategies to tackle respiratory viruses are of a high priority and in this lecture our engagement of structure-guided antiviral drug discovery will be presented. Using the structures of target proteins that are critical in the virus' lifecycle and targeting highly-conserved domains within these proteins, we have been able to develop potent inhibitors of influenza virus and human parainfluenza virus. Employing an integrated interdisciplinary approach that combines, structural and computational biology, chemistry and virology we have explored the active sites of neuraminidase and haemagglutinin-neuraminidase from influenza virus and human parainfluenza virus, respectively. Some of our unpublished preliminary data using fragment-based structure-guided drug discovery will also be presented.

005. Inhibiting Entry of Human Paramyxoviruses

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Infection by paramyxoviruses, with the prime example of human parainfluenza virus, causes a global disease burden and a wide spectrum of respiratory illnesses. Parainfluenza virus enters cells by fusing its envelope directly with the cell membrane in a process mediated by the viral surface glycoproteins HN (receptor-binding protein; hemagglutinin-neuraminidase) and F (fusion protein) — together forming the "fusion complex." To successfully infect, the HN-F complex must first be prevented from activating prematurely in the absence of its cellular target, and second undergo a precise set of activation steps at the right time and place. Upon engagement of a cellular receptor, HN triggers F to undergo a series of structural transitions, inserting in the target membrane and refolding to mediate virus-cell fusion. These two phases are finely tuned to fit human cells and rapidly evolve depending on context. Each step of HN-F interaction presents an attractive anti-viral target, since either premature activation of F or inhibition of subsequent HN-F interaction and the conformational transitions in F will halt infection. In a new broad-spectrum antiviral strategy based on inhibiting fusion during viral entry, we combine structure-based optimization of fusion inhibitory peptides, backbone modification via partial replacement of α-amino acid residues with unnatural β-amino acid residues to enhance inhibitor half-life, and addition of engineered lipid components to develop paramyxovirus fusion inhibitors. Using cryo-electron tomography we characterize the viral fusion complex on the surfaces of viruses before and during engagement of host cell membranes, to uncover new fundamental antiviral targets.

006. Inducing Phase Transition in Viral Condensates.A Novel Antiviral Strategy

Ralf Altmeyer, Ph.D.[1], Jennifer Risso-Ballester, Ph.D.[2], Marie Galloux, Ph.D.[3], Ronan Le Goffic, Ph.D.[3], Charles-Adrien Richard, Ph.D.[3], Fortune Hontonnou, Ph.D.[3], Jean-François Eléouët, Ph.D.[3], Marie-Anne Rameix-Welti, M.D., Ph.D.[2]

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Biomolecular condensates have emerged as an important subcellular organizing principle. Viruses use liquid-liquid phase separated condensates to conduct essential replication functions such as genomic and messenger RNA synthesis. Replication of human respiratory syncytial virus (RSV) occurs in virus-induced condensates called inclusion bodies (IBs). We recently showed that RSV IBs are biomolecular condensates that form through phase separation. We have identified that the steroidal alkaloid cyclopamine and its chemical analogue A3E inhibit RSV replication by disorganizing and hardening IB condensates. Viral condensates are selective drug targets as they are (1) not needed by the host cells, and (2) composed of only a small number of viral proteins. The actions of cyclopamine and A3E were blocked by a point mutation in the RSV transcription factor M2-1. IB disorganization and loss of liquid properties occurred within minutes, suggesting that the compounds act directly on proteins, PPIs or protein-nucleic acid interactions which are required to maintain the liquid properties of the IB condensate. A3E and cyclopamine inhibit RSV in the lungs of infected mice providing proof of concept that targeting pathological condensates can translate to therapeutic benefit. We believe that targeting the liquid state of pathological condensates will enable fast pharmacological modulation of viral replication in patients infected with viruses causing acute respiratory infections. Risso-Ballester et al., Nature. 2021 Jul;595(7868):596-599. doi: 10.1038/s41586-021-03703-z. Bailly et al., Sci Rep. 2016. doi: 10.1038/srep25806.

007V. Monoclonal Antibodies against both Flu and RSV

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The ongoing COVID-19 is not the only respiratory infection causing concern. Influenza (flu) and respiratory syncytial virus (RSV) also pose serious risks to public health. Rapid emergence of new subtypes of human-infecting avian influenza viruses like H7N9, H7N4, H5N1, H5N6, H5N8 and H10N3, calls for effective broadly neutralizing antibodies or vaccines for the controls prior to their outbreaks. In addition, the identification of bat-origin subtypes H17N10 and H18N11 makes the ecological cycle of influenza viruses more complicated. Identification of broadly neutralizing/protective antibodies against distinct subtypes of influenza viruses, has long been a priority and has renewed hopes of generating universal vaccines. HA and neuraminidase (NA), the two surface glycoproteins of influenza virus, are key targets for antibody development. I will present several human broadly neutralizing HA stem-specific antibodies with distinct binding modes. One of them, could also inhibit bat-origin H17 and H18 HAs-mediated membrane fusion induced by low pH, indicating its encouraging translational applications for both endemic and emerging potential influenza viruses. I will also introduce an anti-NA human antibody that could restore protection efficacy against the drifted influenza virus bystructure-based modification. RSV is the leading causative agent for infant hospitalizations with lower respiratory infections. The only approved prophylaxis is the humanized monoclonal neutralizing antibody palivizumab for infants at high risk. I will present the isolation of a human monoclonal antibody, RV11, that targets prefusion state of viral fusion protein, with neutralizing activity superior to palivizumab. Our study provided a promising candidate that add the arsenal against RSV infection.

008. Treatment of EV-D68 Respiratory and Neurological Disease in AG129 Mice with a Monoclonal Antibody, EV68-228

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In 2014, 1,395 cases of respiratory disease caused by enterovirus D68 (EV-D68) in 49 states and the District of Columbia were confirmed by Centers for Disease Control and Prevention (CDC) and public health laboratories. In addition to respiratory disease, the outbreak of EV-D68 was associated with cases of acute flaccid myelitis (AFM) cases characterized by muscle weakness and paralysis. In 2018, more than 90% of patients with AFM had a mild

respiratory illness or fever prior to developing AFM. As the CDC has recognized that enteroviruses likely play a role in AFM, we evaluated a monoclonal antibody (MAb), EV68-228, for treatment of EV-D68 respiratory and neurological infections in an AG129 mouse model. In the respiratory model of disease, a single intraperitoneal administration of EV68-228 24 hours after infection reduced lung and blood virus titers to below the limit of detection. In addition, concentrations of pro-inflammatory lung cytokines were significantly reduced by treatment with EV68-228. A single treatment with EV68-228 at a dose of 1 mg/kg administered 48 hours post-infection reduced lung and blood virus titers as well as pro-inflammatory lung cytokines. In the neurological model of disease, a single treatment with EV68-228 at a dose of 1 mg/kg administered 24 hours post-infection completely protected mice from mortality and paralysis. A single treatment with 10 mg/kg of EV68-228 protected four of six mice from mortality when administered 48 hours post-infection.

[Supported by Contract HHSN272201700041I from the Virology Branch, DMID, NIAID, NIH and grant U19 AI117905 from DAIT, NIAID, NIH].

009. Impact of PA E23G/K Substitutions on Influenza A Virus Fitness and Baloxavir Susceptibility

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Baloxavir marboxil (BXM) and its active metabolite baloxavir acid (BXA) are potent influenza antivirals. Treatment-emergent reduced-susceptibility substitutions occur at PA I38T and E23G/K, but the latter are insufficiently described. We generated A(H1N1)pdm09 or A(H3N2) influenza viruses with E23G/K +/- I38T. Viruses with E23G/K displayed a 2 to 13-fold increase in BXA EC_{50}s vs. wild-type (WT), but 7 to 33-fold decrease in EC_{50}s vs. I38T. Combining E23G/K and I38T increased EC_{50}s 138 to 446-fold vs. WT, and 2 to 6-fold vs. I38T. Replication, polymerase assays, and plaque diameters showed E23K viruses were more fitness impaired in cell culture than E23G, with E23K+I38T being the most impaired. In the ferret transmission model, all tested viruses transmitted to direct and airborne ferret contacts, though the virus with dual E23K+I38T failed to achieve 100% airborne transmission. Sequence analysis showed E23G/K +/- I38T genotypes were stable during these transmission events. To understand the mechanism of E23G/K BXA reduced susceptibility, recombinant PA endonuclease domain (PA_N)-BXA protein interactions were measured by a modified thermofluor assay. Thermo-stable PA_N-BXA interactions were decreased by the acquisition of E23G/K substitutions and further decreased by addition of I38T. In summary, while E23G/K substitutions negatively affect in vitro fitness, they do not alter transmissibility or genetic stability. The synergistic effect of E23G/K+I38T dramatically elevated EC_{50}s more than the sum of the individual substitutions. Decreased drug-protein interactions partially explains reduced BXA susceptibility caused by E23G/K. These data suggest E23G/K or dual mutations with I38T should be considered important BXM reduced-susceptibility markers in antiviral surveillance.

010. Targeted Protein Degradation as an Antiviral Strategy

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We currently do not have effective vaccines or antiviral drugs for most of the viral diseases that afflict humans. Antiviral therapies that enable long-term control over human immunodeficiency virus (HIV) infection and cure chronic hepatitis C virus (HCV) have been landmark successes in the treatment of viral infections. These therapies work through the use of multiple direct-acting antiviral (DAA) drugs that act by potently inhibiting the function of viral proteins, generally enzymes such as viral polymerases and proteases. Narrow spectrum activity (i.e., the "one bug, one drug" problem) and drug resistance are two major challenges for the antivirals field. This is especially true against viruses with RNA genomes because limited or no proofreading function of the viral polymerase generates tremendous genetic diversity. There is thus a need for antiviral strategies that make use of alternative targets, mechanisms, and modalities to combat viral diseases and to avoid antiviral resistance. My group has focused on the discovery and validation of new antiviral targets and strategies. Here I hope to provide to examples of our proof-of-concept work establishing targeted protein degradation as an alternative antiviral strategy.

011. Cell-to-Cell Transmission by Emerging Viruses: Mechanisms of Action and Evasion of Host Immunity

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Emerging and re-emerging viruses spread globally without borders. In the past 40 years, the world has witnessed outbreaks of HIV/AIDS, SARS, MERS, Ebola, Zika, and more recently COVID-19. SARS-CoV-2 is a novel coronavirus, which likely evolves from viruses in bats and spills over to humans through intermediate animal species. While much has been studied on SARS-CoV-2 entry and infection, less is known about the impact of cell-to-cell transmission on virus spread and immune responses. In this talk, I will describe our recent work on SARS-CoV-2 spike-mediated cell-to-cell transmission in comparison with that of SARS-CoV-1 and in the context of cell-free infection as well as variants of concern (VOCs). I will show evidence that SARS-CoV-2 spike is more efficient in facilitating cell-to-cell transmission than is SARS-CoV-1 spike, which reflects, in part, their differential cell-cell fusion activity. Interestingly, we find that treatment of cocultured cells with endosomal entry inhibitors impairs cell-to-cell transmission, implicating endosomal membrane fusion as an underlying mechanism. Compared with cell-free infection, cell-to-cell transmission of SARS-CoV-2 is refractory to inhibition by neutralizing antibody or convalescent sera of COVID-19 patients. While ACE2 enhances cell-to-cell transmission, we find that it is not absolutely required. Notably, despite differences in cell-free infectivity, the authentic VOCs B.1.1.7 (alpha) and B.1.351 (beta) have similar cell-to-cell transmission capability. However, the Omicron variant (B.1.1.529) exhibits significantly increased capability of cell-to-cell transmission. The significance and implications of these findings for SARS-CoV-2 spread and pathogenesis will be discussed.

012. Lenacapavir: The First Clinically Active Long-Acting Inhibitor of HIV Capsid

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A program building on prior extensive structural and functional characterization of HIV capsid and spanning a decade of drug discovery work yielded Lenacapavir (LEN, GS-6207), a small molecule inhibitor targeting several critical functions of HIV capsid. LEN binds at a conserved interface between capsid monomers and interferes with protein interactions essential for multiple phases of HIV replication cycle including both assembly and disassembly of capsid core, as well as capsid nuclear trafficking. LEN exhibits antiviral activity at picomolar concentrations against all subtypes of HIV, including strains resistant to other antiretroviral classes, and is amenable to both oral and long-acting subcutaneous administration. In a Phase 1 study in treatment-naïve people with HIV, LEN (50 mg to 750 mg) showed a rapid and dose-dependent antiviral effect, with up to 2.3 mean $\log_{10}$ decrease in HIV-1 RNA at day 10. In people with multi-drug-resistant HIV, subcutaneous LEN administered every 6 months in combination with optimized background regimen led to high rates of virologic suppression and was well tolerated. Finally, emerging data from non-human primates support further investigation of LEN as a long-acting agent for pre-exposure prophylaxis.

013. Antiviral Therapy for Lassa Fever

Elisa Pallasch, B.S.[1], Sabrina Bockholt, B.S.[1], Jonas Müller, B.S.[1], Stephan Günther, M.D., Ph.D.[1],
Lisa Oestereich, Ph.D.[1]
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Lassa fever (LF) is a zoonotic viral infection caused by Lassa virus (LASV), an Old World arenavirus. It is assumed to be responsible for up to three hundred thousand cases and more than five thousand deaths annually, with fatality rates ranging from 20% to 70% in hospitalized cases. LF is endemic in many West-African countries and its epidemic potential and the lack of therapeutics or vaccines leads to its classification as a priority pathogen by the WHO. Severe LF may be associated with haemorrhage, oedema of the face and neck, liver damage, kidney dysfunction, and central nervous system manifestations such as coma and seizures. But despite the high morbidity and mortality caused by LASV infections, very little is known about the pathophysiology of the disease. Studies with human samples revealed that a dysregulation of homeostasis, strong inflammation and vascular dysfunction are hallmarks of Lassa fever (LF). To gain a better understanding of the pathophysiology of LF, we developed a susceptible mouse model with intact adaptive, hematopoietic-driven immune response. We used this mouse model to describe that T cell-mediated immunopathology plays an important role during LF. Based on these results, we applied immune-modulatory drugs for experimental treatments in mice and could identify two compounds that were able to reduce the disease symptoms to a remarkable degree, indicating that the modulation of T cell responses might be an approach for the treatment of LF.

014. Prophylactic Treatment with a Defective Interfering Particle-Based Therapeutic Protects Hamsters from Lethal Nipah Virus Disease Primarily by Direct Inhibition Mechanisms

Stephen Welch, Ph.D.[1], Jessica Spengler, Ph.D.[1], Jessica Harmon, M.S.[1], JoAnn Coleman-McCray, B.S.[1], Sarah Genzer, D.V.M.[1], Teresa Sorvillo, Ph.D.[1], Florine Scholte, Ph.D.[1], Michael Lo, Ph.D.[1], Joel Montgomery, Ph.D.[1], Stuart Nichol, Ph.D.[1], Christina Spiropoulou, Ph.D.[1]

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Genomes of defective interfering particles (DIs) are considerably truncated compared to the parental virus and generally lack any protein coding capacity, but importantly retain their replication capability. When DIs are present alongside their parental virus, their propagation can negatively impact the latter's growth kinetics, and exert potent inhibitory properties. We previously reported on both the in vitro and in vivo therapeutic potential of DIs when co-administered with challenge virus. To further characterize their potential for short-course prophylactic and post-exposure administration, we investigated various clinical and virological parameters in the lethal Syrian hamster model of NiV disease when DIs were provided at various timepoints up to a week prior to infection, or one day post-infection. In these studies, we found that a DI based on NiV strain Malaysia could confer up to 90% survival against homologous virus challenge when administered prophylactically, as well as significantly reduce the appearance and length of clinical signs. The relative contribution of direct and indirect inhibition elicited by DIs in vivo remains unclear, and the non-specific immunostimulatory effect of DIs have been suggested to contribute significantly to protective efficacy. However, when we assessed treatment against both a different strain of NiV (strain Bangladesh) and also the closely related henipavirus Hendra virus, efficacy was significantly decreased, supporting direct interference as a primary mechanism of action. These data further support putative therapeutic applications for DI particles, and encourage further development of DI therapeutics for NiV and for other high-consequence pathogens.

015. Small Molecule Antivirals Inhibit HuNoV GII.4 in Human Intestinal Enteroids

Nanci Santos-Ferreira, M.S.[1], Carmen Mirabelli, Ph.D.[2], Jonathan Sexton, Ph.D.[3], Johan Neyts, Ph.D.[1], Christiane Wobus, Ph.D.[2], Joana Rocha-Pereira, Ph.D.[1]

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Human Norovirus (HuNoV) is the main cause of acute gastroenteritis resulting in high morbidity and mortality, especially in children under 5 years old. Unavailability of antiviral drugs or vaccines is mostly due to the historic lack to propagate HuNoV. Human intestinal enteroids (HIEs) have emerged as a unique model to culture HuNoV *in vitro*. To setup an antiviral assay using HIEs, HuNoV replication was evaluated in 2D monolayers or 3D cultures of differentiated HIEs from adult jejunum (J2) and fetal ileum (FI124) at 2-3 days postinfection by RT-qPCR. Replication of HuNoV GII.4 strains reached higher yields in the FI124 line and more reproducible infections in 3D, attaining an average increase over input of 3.8 $\log_{10}$ in HuNoV genome equivalents. Treatment of GII.4 3D infected FI124 enteroids with a non-toxic concentration of 50 μM of the polymerase inhibitor 2'-C-Methylcytidine (2CMC) after infection showed a reduction of 1.3 $\log_{10}$ in HuNoV genome equivalents. When the drug was added during infection a reduction of 2.0 $\log_{10}$ viral RNA copies was achieved. Other antivirals that showed activity in this model included nitazoxanide, favipiravir and rupintrivir. We have thus validated this antiviral assay using a qPCR-readout, and are currently adapting the assay to a high content imaging readout by staining virus and host cells with specific antibodies. Overall, 3D infection of FI124 HIEs is a robust model to evaluate antivirals that will now be used to assess the antiviral activity of novel compounds against HuNoV GII to significantly advance antiviral research for norovirus disease.

016. Fleximers – A Strategic Approach to Broad-spectrum Antiviral Therapeutics

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Over the past several decades nucleos(t)ides have maintained a prominent role as one of the cornerstones of antiviral and anticancer therapeutics. As a result, numerous approaches to nucleos(t)ide and nucleic acid drug design have been pursued. One such approach involves adding flexibility to the sugar moieties of nucleos(t)ides, for example, in the highly successful anti-HIV/HBV drug Tenofovir developed by Antonín Holý. In contrast, introduction of flexibility to the nucleobase scaffold has only more recently gained significance with the invention of our fleximers. This modification has led to a significant improvements in antiviral activity, and in some cases endowing the nucleoside with potent activity when the parent rigid nucleoside was inactive. Another advantage observed is the ability to avoid resistance mechanisms related to point mutations by engaging secondary amino acid residues not previously involved in the mechanism of action. The history of their development, and recent (and unexpected!) antiviral findings for this innovative class of nucleos(t)ides will be discussed.

017. Out of the Box: 20 Years of Targeting Virus Infections Unconventionally

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Over the past two decades, my science has addressed virus biology on the molecular level, relying on some bigger picture concepts stemming from evolutionary biology. We use viruses as model organisms to figure out how they work, and then we try to turn the tables on viruses, using what we've learned to target them in new antiviral or vaccine approaches. This presentation will pick examples from over a dozen viruses and several animal models to try and convey our admiration for virus populations as evolving organisms, all the while trying to take them down as public health threats. We'll discuss the coronas, the alphas, the flavis, the picornas and some Flu, and the new antiviral strategies that have stemmed from our research. Marco Vignuzzi obtained his B.Sc from McGill University, and MSc and PhD from University of Paris. Following 7 years of postdoctoral studies in Raul Andino's lab at UCSF, he founded his own laboratory at Institut Pasteur in 2008. Since that time, his work has focused on emerging viral diseases. Marco is on the editorial board of J Virology, Virus Evolution and PLOS Pathogens. He is the co-director of France's LABEX on Emerging Infectious Diseases, that oversees research program funding for 65 French laboratories that cover every emerging threat of viral, bacterial, fungal and parasitic nature. In 2015 his work in emerging diseases was awarded the Sanofi Junior Award in Biomedical Research; in 2019 he received the international Richard Elliott Memorial Award for his work on zoonotic viral diseases. In 20 his lab re-opens its doors at IDLabs within the Agency for Science, Technology and Research in Singapore.

018V. The SARS-CoV-2 Main Protease: New Inhibitors, Crystal Structures, and Mutations

Rolf Hilgenfeld, Ph.D.[1], Kaixuan Zhang, M.S.[1], Judith Roeske, M.S.[1], Linlin Zhang, Ph.D.[1], Matthias Goehl, Ph.D.[2], Mark Broenstrup, Ph.D.[2], Katharina Rox, Ph.D.[2], Yuri Kusov, Ph.D.[1], Ravikumar Akula, Ph.D.[1], Haifa El Kilani, Ph.D.[1], Mohamed Ibrahim, Ph.D.[1], Xinyuanyuan Sun, M.S.[1]
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In February 2020, we determined the crystal structure of the SARS-CoV-2 Mpro and presented a powerful alpha-ketoamide inhibitor, compound 13b [Zhang et al., Science, 2020]. Using a structure-based approach, we have since optimized this compound further and now have inhibitors with IC50 down to 13 nM in the biochemical assay and EC50 < 400 nM in virus-infected cell culture. ADME and pharmacokinetic data will be discussed for the frontrunner compounds. In preparing for future drug resistance mutations, which will likely emerge when the presently available SARS-CoV-2 Mpro inhibitor nirmatrelvir [Owen et al., Science, 2021] and potential future candidate compounds such as 13b-K [Cooper et al., to be submitted] will be used in the clinic, we analyzed the natural evolution of the Mpro since the beginning of the pandemic. When cumulated, the most common mutations are L89F and K90R and we determined crystal structures for both. However, the frequency of L89F is declining and others are coming up, and we will also present structural studies on the most interesting ones. Most mutations are far from the substrate-binding site and the dimerization interface of the enzyme and the inhibitory potency of compound 13b-K is not affected. The Mpro mutation P132H is characteristic for the Omicron variant of concern of SARS-CoV-2 and we will present data on this protein as well. This work was supported, in part, by the BMBF (directly and through DZIF) and by the European Commission.

019. Host-targeting Broad-spectrum Antivirals for Pandemic Preparedness

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The use of a small molecule inhibitor of host cell farnesyltransferase to treat hepatitis delta virus (HDV) infections is an exemplar of key benefits of host-targeting antivirals for pandemic preparedness: 1) because the targeted genetic locus is not under control of the virus, a high barrier to the development of resistance is predicted (and empirically confirmed in phase 2 HDV trials); 2) because multiple viruses often depend on the same host cell function, there is broad-spectrum antiviral potential; 3) because the targeted host function can be relevant in non-viral diseases, there can be additional options for financing development to an advanced state of readiness (i.e. develop for non-viral indication; available for off-label use in a pandemic). There are multiple other attractive host targeting agents. One example is PI4KIIIB inhibitors currently in development for severe enterovirus infections, but broadly active against rhinovirus, SARS-CoV-2, etc., and also useful to treat numerous cancers/metastasis. Finally, type III interferon lambda represents an ideal host-targeting broad-spectrum antiviral for pandemic viruses. Lambda is the body's first line of defense against viruses and has similar antiviral activity to traditional type I interferon like interferon alpha–but lambda is much better tolerated. Alpha has widely distributed receptors, including on immune cells, which can exacerbate a cytokine storm. Indeed, alpha given after a lethal flu inoculum exacerbates disease and mortality; whereas lambda gives dramatically improved survival. Lambda is broadly active against numerous viruses in vitro and in animal models (e.g. SARS-CoV-2, influenza, etc.), has a well-established tolerability profile in over 3000 patients in the context of over 20 clinical trials, and is highly effective against SARS-CoV-2, which has led to phase 2 and 3 studies, with unparalleled efficacy following a single subcutaneous dose. Taken together, host-targeting, broad-spectrum antivirals represent ideal components of our antiviral toolkit for pandemic preparedness.

020. Efficient Incorporation and Template-Dependent Polymerase Inhibition are Major Determinants for the Broad-Spectrum Antiviral Activity of Remdesivir

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Remdesivir (RDV) is a direct-acting antiviral agent that is approved in several countries for the treatment of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). RDV exhibits broad-spectrum antiviral activity against positive-sense RNA viruses, e.g. SARS-CoV-2 and hepatitis C virus (HCV) and non-segmented negative-sense RNA viruses, e.g. Nipah virus (NiV), while segmented negative-sense RNA viruses such as influenza (Flu) virus or Crimean-Congo hemorrhagic fever virus (CCHFV) are not sensitive to the drug. The reasons for this apparent pattern are unknown. Here, we expressed and purified representative RNA-dependent RNA polymerases (RdRp) and studied three biochemical parameters that have been associated with the inhibitory effects of RDV-triphosphate (TP): (i) selective incorporation of the nucleotide substrate RDV-TP, (ii) the effect of the incorporated RDV-monophosphate (MP) on primer extension, and (iii) the effect of RDV-MP in the template during incorporation of the complementary UTP. The results of this study revealed a strong correlation between antiviral effects and efficient incorporation of RDV-TP. Inhibition in primer extension reactions is heterogeneous and usually inefficient at higher NTP concentrations. In contrast, template-dependent inhibition of UTP incorporation opposite the embedded RDV-MP is seen with all polymerases. Molecular modeling suggests a steric conflict between the 1′-cyano group of RDV-MP and conserved residues of RdRp motif F. We conclude that future efforts in the development of nucleotide analogues with a broader spectrum of antiviral activities should focus on improving rates of incorporation while capitalizing on the inhibitory effects of a bulky 1′-modification.

021. Countering Pathogenic New World Mammarenavirus Infections Through Receptor-Targeted Disruption of Virus Entry

Brady Hickerson, Ph.D.[1], Cristian Payes, Ph.D.[2], Lars Clark, Ph.D.[3], Kevin Bailey, B.S.[1], Eric Sefing, M.S.[1], Samantha Zink, Ph.D.[4], James Ziegenbein, B.S.[4], Pierre Candelaria, Ph.D.[5], Jonathan Abraham, M.D., Ph.D.[3], Tracy Daniels-Wells, Ph.D.[5], Gustavo Helguera, Ph.D.[2], Manuel Penichet, M.D., Ph.D.[5], **Brian Gowen, Ph.D.[1]**

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Five New World mammarenaviruses (NWMs) can cause a life-threatening hemorrhagic fever (HF) syndrome. Cellular entry by these viruses is mediated by human transferrin receptor 1 (hTfR1). Here, we demonstrate that a mouse/human chimeric antibody (ch128.1/IgG1) which binds the apical domain of hTfR1, potently inhibits infection of attenuated and pathogenic NWAs *in vitro*. Computational docking of the antibody Fab crystal structure onto the known structure of hTfR1 shows an overlapping receptor-binding region shared by the Fab and the viral envelope glycoprotein GP1 subunit that binds hTfR1, and we demonstrate competitive inhibition of NWM GP1 binding by ch128.1/IgG1 as the principal mechanism of action. Importantly, we also found that this antibody-based strategy protects hTfR1-expressing transgenic mice against lethal NWM challenge. Our findings provide the basis for developing a novel, host receptor-targeted antibody therapeutic broadly applicable to the treatment of HF of NWM etiology.

022. Development of Small Molecule Entry Inhibitors as Potential Filoviral Therapeutics

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Filoviruses (Ebola and Marburg virus) cause severe hemorrhagic fever in humans and nonhuman primates. Currently, there are no FDA-approved therapeutics or vaccines to treat filovirus infections. Many small molecules have been identified as entry inhibitors of Ebola virus (EBOV) and Marburg virus (MARV). However, there is a significant lack of understanding on the mechanism of action for these molecules, limiting further development of these inhibitors as anti-filoviral agents. Here we provide evidence that toremifene and other small molecule entry inhibitors have at least three distinct mechanisms of action. First, many small molecules can directly bind to the internal fusion loop region of EBOV glycoprotein (GP). Second, the HR2 domain is the main binding site for MARV GP inhibitors and a secondary binding site for some EBOV GP inhibitors. Finally, lysosomal trapping of GP inhibitors enhances drug concentrations in the lysosome and further improves the viral inhibition. Furthermore, a combination of small molecules targeting different domains of the EBOV GP are synergistic in inhibiting viral entry. Overall, our findings provide mechanistic insights on filovirus entry and rational drug design for future antiviral development.

023. A Photoactivable Chlorophyll-Derived Product with Broad Antiviral Activty against Enveloped Viruses Including Highly Pathogenic Coronaviruses

Thomas Meunier, Ph.D.[1], Lowiese Desmarets, Ph.D.[1], Simon Bordage, Ph.D.[2], Moussa Bamba, Ph.D.[2], Kevin Hervouet, Ph.D.[1], Yves Rouille, Ph.D.[1], Nathan Francois, B.S.[1], Marion Decossas, M.D.[3], Valentin Sencio, Ph.D.[1], Francois Trottein, Ph.D.[1], Fezan Tra Bi, Ph.D.[4], Olivier Lambert, Ph.D.[3], Jean Dubuisson, Ph.D.[1], Sandrine Belouzard, Ph.D.[1], Sevser Sahpaz, Ph.D.[2], **Karin Seron, Ph.D.[1]**

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The COVID-19 pandemic has highlighted the lack of specific antiviral compounds available against coronaviruses (CoVs). Bioguided fractionation of Ivorian plant extracts identified pheophorbide a (Pba) as a highly active antiviral molecule against HCoV-229E in cell culture. The antiviral activity of Pba was subsequently shown for the highly pathogenic coronaviruses SARS-CoV-2 and MERS-CoV in cell culture. The study of the mechanism of action revealed that Pba is an inhibitor of coronavirus entry by directly targeting the viral particle. Interestingly, the antiviral activity of Pba depended on light exposure and on the generation of singlet oxygen, revealing a photodynamic inactivation mechanism. Pba inhibited virus-cell fusion by stiffening the viral membrane as demonstrated by cryo-electron microscopy. Moreover, Pba was shown to be broadly active against several other enveloped viruses, but not against

non-enveloped virus. Finally, Pba was able to reduce SARS-CoV-2 and MERS-CoV replication in primary human bronchial epithelial cells. The antiviral activity of Pba was also studied in an ACE2-humanized mouse model infected with SARS-CoV-2. Pba is the first described natural antiviral against SARS-CoV-2 with direct photosensitive virucidal activity that holds potential for COVID-19 therapy, disinfection of SARS-CoV-2 contaminated surfaces, veterinary medicine and many other applications.

024. A Broad-Spectrum Ribonucleoside Analog, EIDD-2749, Provides Protection Against Enterovirus D68 and 71 Infections in Mouse Models

E. Bart Tarbet, Ph.D.[1], Brett Hurst, Ph.D.[1], Manohar Saindane, Ph.D.[2], Alexander Kolykhalov, M.D., Ph.D.[2], George Painter, Ph.D.[2], Gregory Bluemling, Ph.D.[2]

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A broad-spectrum ribonucleoside analog, EIDD-2749 discovered at the EIDD and currently being developed by DRIVE, was evaluated against Enterovirus D68 (EV-D68) and Enterovirus 71 (EV-71) infections in mice. In the EV-D68 respiratory model in 4-week-old AG129 mice, oral treatment with EIDD-2749 significantly reduced lung virus titers in a dose-dependent manner. A dose of 30 mg/kg/day reduced lung virus titers by 1000-fold at days 1, 3, and 5 post-infection compared to placebo-treated mice. EIDD-2749 treatment also reduced viremia at days 1, 3, and 5 post-infection. No virus was detected in the blood of animals treated with a dose of 30 mg/kg/day. Infection-associated inflammation in the lung was reduced by treatment with EIDD-2749, as indicated by reduced lung concentrations of IL-1α, IL-1β, IL-6, MCP-1, and RANTES. In the EV-D68 neurological model, i.p. treatment with EIDD-2749 at 6 or 20 mg/kg/day provided 100% protection from neurological signs and mortality after infection of 10-day-old mice. In addition, oral treatment with EIDD-2749 at doses of 3, 10, and 30 mg/kg/day protected mice from mortality and weight loss caused by EV-71 infection in 4-week-old mice and significantly reduced neurological scores in a dose-dependent manner. A fixed dose of 10 mg/kg/day of EIDD-2749 completely protected mice from weight loss and mortality when treatment started 3, 5, or 7 days after infection, and protected mice from paralysis as measured by neurological score. These results demonstrate EIDD-2749 therapeutic efficacy against EV-D68 and EV-71 infections in mouse models.

[Supported by NIH contracts HHSN272201000040I and HHSN272201500008C, and DOD contract HDTRA1-15-C-0075]

025. Antiviral Activity of Geneticin Against SARS-CoV-2

Gregory Mathez, M.S.[1], Carmine Varricchio, Ph.D.[2], Caroline Tapparel, Ph.D.[3], Laurent Kaiser, M.D.[4], Andrea Brancale, Ph.D.[2], Valeria Cagno, Ph.D.[1]

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SARS-CoV-2 pandemic is currently causing an unprecedented impact on our society. Vaccines are largely deployed but efficient large-scale antiviral therapies are still lacking. Geneticin, an aminoglycoside antibiotic known to interact with ribosomal RNA, showed broad-spectrum activity against RNA viruses, in particular dengue virus and hepatitis virus, at conditions not showing cellular toxicity, through interaction with double-stranded structures in the viral RNA. SARS-CoV-2 has as well conserved secondary structures, especially a ribosomal frameshifting signal between ORF1a and ORF1b. Therefore, we investigated if geneticin is exerting antiviral activity against SARS-CoV-2. Geneticin showed efficacy in vitro, at nontoxic doses, against different variants of SARS-CoV-2 with EC50s in the range of 16 to 64 µg/mL and efficacy in human lung cells and human-derived respiratory tissues. Kinetics experiments showed early inhibition with reduced expression of viral protein and the level of viral RNA. We did not observe resistance to geneticin so far. In silico models showed interaction with the SARS-CoV-2 pseudoknot necessary for the -1 programmed ribosomal frameshifting. We confirmed this interaction with a dual luciferase system that showed a decrease in frameshift efficiency. From a virtual screening of a library of RNA binders commercially available, we identified several compounds, which showed efficacy in the micromolar range validating as well the mechanism of action. Further work will be directed toward selecting analogs with increased antiviral activity.

026. Automated Brightfield Microscopy and AI to Develop Rapid High Throughput Infectivity Assays for Screening Antiviral Drugs and Monitoring Vaccine Effectiveness against Emerging Variants

Rupert Dodkins, Ph.D.[1], Tess Overton, B.S.[2], John Delaney, M.S.[1], Kathy Yeung, M.B.A.[1], Frank Scholle, Ph.D.[2], **Ilya Goldberg, Ph.D.**[1]

[1]ViQi Inc, Santa Barbara, California, United States of America; [2]NC State University, Raleigh, North Carolina, United States of America

Viral infectivity assays are essential for the development of viral vaccines and anti-viral drugs. Plaque and TCID50 assays can take as long as 2-14 days to develop. Alternatives like fluorescent focus assay (FFA) are faster but require staining or GFP-labeled viruses. ViQi, Inc. has developed a quantitative viral infectivity assay using machine learning and brightfield microscopy to identify the phenotypic changes within cells associated with viral production before being humanly visible. As with FFA, these changes can be detected within a few hours after infection depending on the virus, but do not require any sample preparation. Each virus requires training a new AI and only needs to be done once to establish and validate the assay. Once an AI is trained, assays can be processed within hours of infection, producing same-day or next-day results. This technology promises to reduce turn-around time, increase reliability, lower labor costs, ease automation, and increase throughput 10-fold or more. These can be substantial advantages for BSL-3 class viruses. We have trained AI models on seven viruses so far including enveloped, non-enveloped, DNA and RNA viruses. In each instance, the dilution calibration curve yielded an R2 of no lower than 0.9, and the linear range of the assay was typically equivalent or more broad than that of plaque assays, which is typically readable over a 10-fold range. Because this technology relies on AIs sensitized to potentially specific phenotypes of viral production, it may have potential as a rapid, broad-based identification and diagnostic tool.

027. Fighting Viral Hemorrhagic Fevers: From the Benches to the Trenches

Christina Spiropoulou, Ph.D.[1]

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In this talk I would like to highlight the contribution of my team over the years in the area of viral hemorrhagic fever (VHF) antiviral discovery. I've been working on VHFs for the last 30 years, both as a molecular virologist and as a public health scientist. VHF viruses comprise a very diverse collection of mostly high consequence/low incidence zoonotic RNA viruses including viruses such as Ebola, Nipah, Lassa and Crimean-Congo hemorrhagic fever viruses. Their high diversity and low incidence create a considerable challenge for the study of these viruses, from the characterization of the virus life cycle to creation of animal models of disease, to the development of therapeutics and vaccines. Much of our work focuses on using reverse genetics to build reporter-tagged viruses useful for tracking virus infections in animal models to identify potential drug targets and for high-throughput antiviral screening assays. In addition, reverse genetics engineered VHF viruses with strategic genome deletions to inhibit their spread show promise as safe and efficacious prototype vaccines. I will highlight the most promising therapeutic candidates in various categories that we have seen to date, including remdesivir, which made it through the pipeline to clinical trials for Ebola treatment and most recently is being investigated for clearing virus persistence infections which can lead to initiation of new Ebola virus outbreaks. The COVID-19 pandemic has made it clear to everyone the importance of developing medical countermeasure tools to combat emerging virus threats and prepositioning these prior to global crises arising. I remain excited to try to continue to contribute to this important area.

028. Antiviral Strategies for Arboviruses: A 'Buzzing' Role for the Mosquito Vector?

Leen Delang, Ph.D.[1]

[1]University of Leuven, Leuven, Belgium

Mosquitoes are recognized as the deadliest animals on earth. Their bites result in the death of almost 1 million people every year due to pathogens that are transmitted via the mosquito saliva. These pathogens include the diverse group of arboviruses (i.e. arthropod-borne viruses). Arboviruses can cause severe diseases such as hemorrhagic fever, encephalitis and chronic arthritis. Despite their significant disease burden and widespread presence, there are currently no antivirals available for treatment. As the arbovirus viremia is rather short, there is only a small window for therapeutic intervention. Therefore, novel strategies might be needed to complement traditional antiviral therapies and mosquito-control methods. A new approach could be the use of antiviral drugs to block arbovirus replication in the mosquito. A possible route by which a mosquito could take up an antiviral drug, is through the ingestion of blood

from a patient that is being treated with the drug. Antiviral activity in the mosquito could be favorable, since this might prevent further transmission to vertebrate hosts. On the other hand, drug-resistant virus variants could emerge in the mosquito when exposed to antiviral drugs. By mosquito infection studies, we have shown that favipiravir was not able to decrease the replication of chikungunya virus in *Aedes aegypti* mosquitoes. In contrast, the pan-serotype dengue virus inhibitor JNJ-A07 significantly reduced dengue virus infection in mosquitoes. Moreover, virus dissemination to the salivary glands was completely inhibited by the drug, suggesting that exposure of mosquitoes to a potent antiviral could majorly decrease virus transmission by mosquitoes.

029. New Insights into Cellular Infection by the Mosquito-Transmitted Rift Valley Fever Virus

Amy Hartman, Ph.D.[1]

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Rift Valley Fever Virus (RVFV) is a zoonotic pathogen with pandemic potential that can impact both human and animal health. RVFV entry is mediated by the viral glycoprotein n (Gn), but host receptors and other entry factors remain poorly defined. We identified low-density lipoprotein receptor-related protein 1 (Lrp1) as a critical host factor for entry and infection by multiple strains of RVFV. RVFV Gn directly binds to Lrp1 at specific clusters in a glycosylation-independent manner. Exogenous addition of the Lrp1-binding chaperone protein RAP or anti-Lrp1 antibodies neutralize RVFV infection in evolutionarily distinct cell lines. *In vivo* relevance is highlighted by the finding that mice treated with RAP are protected from disease and death after infection with pathogenic RVFV. Altogether, these data support Lrp1 as a critical host entry factor for RVFV infection and provides a new target for therapeutic antibodies to limit RVFV infections. The use of Lrp1 for entry of RVFV will be discussed in a broader context of bunyavirus tropism and biology.

030. NITD-688, A Pan-Serotype Inhibitor of the Dengue Virus NS4B Protein, Shows Favorable Pharmacokinetics and Efficacy in Preclinical Animal Models

Feng Gu, Ph.D.[1]

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Dengue virus (DENV) is a mosquito-borne flavivirus that poses a threat to public health, yet no antiviral drug is available. We performed a high-throughput phenotypic screen using the Novartis compound library and iden- tified candidate chemical inhibitors of DENV. This chemical series was optimized to improve properties such as anti-DENV potency and solubility. The lead compound, NITD-688, showed strong potency against all four sero- types of DENV and demonstrated excellent oral efficacy in infected AG129 mice. There was a 1.44-log reduction in viremia when mice were treated orally at 30 milligrams per kilogram twice daily for 3 days starting at the time of infection. NITD-688 treatment also resulted in a 1.16-log reduction in viremia when mice were treated 48 hours after infection. Selection of resistance mutations and binding studies with recombinant proteins indicated that the nonstructural protein 4B is the target of NITD-688. Pharmacokinetic studies in rats and dogs showed a long elimination half-life and good oral bioavailability. Extensive in vitro safety profiling along with GLP rat and dog toxicology studies showed that NITD-688 was well tolerated after 14-day repeat dosing, demonstrating that NITD-688 may be a promising preclinical candidate for the treatment of dengue.

031. AI-Derived Antibody Discovery – Humanoids for Global Good

Randal Ketchem, Ph.D.[1]

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Ideally, antibody therapeutics are both efficacious and developable. Antibody discovery platforms tend to focus on either in vivo immunizations or libraries of randomized CDRs on germline frameworks. While immunizations lead to high affinity and specificity, B-cells have no selective pressure to produce developable antibodies. Standard libraries generally do not represent actual human response. We have developed an AI-generated antibody library platform utilizing a Generative Adversarial Network (GAN) which has the ability to generate novel sequences which mimic natural human response as well biasing toward diversity, desired efficacy, and developability features. The resulting libraries have been shown to be successful in obtaining active antibodies.

032. Dengue Virus Infection and Dissemination in Aedes Mosquitoes is Significantly Reduced Upon Exposure to JNJ-A07, A Potent DENV Inhibitor, In The Blood Meal

Ana Rosales Rosas, M.S.[1], Lanjiao Wang, Ph.D.[1], Suzanne Kaptein, Ph.D.[1], Olivia Goethals, Ph.D.[2], Leen Delang, Ph.D.[1]

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Dengue virus (DENV) is the most widespread mosquito-borne virus worldwide; however, no antiviral therapies are available yet. The pan-serotype DENV inhibitor (JNJ-A07) has shown potent activity in a mouse model (Kaptein et al, Nature 2021). As a prophylaxis, this drug could prevent human infection after the bite of an infected mosquito. Moreover, infected mosquitoes might ingest the drug during blood feeding. It is currently unknown whether an antiviral drug ingested by mosquitoes could inhibit virus replication, and thus reduce virus transmission to other hosts. Here, we investigated the antiviral activity of JNJ-A07 administered via a DENV-infectious blood meal to Aedes aegypti mosquitoes.

We first demonstrated that a high concentration of JNJ-A07 (100 µM) had no detrimental effect on the mosquitoes' lifespan. Egg development however was slightly increased (1.2-fold). As a proof of concept, mosquitoes were exposed to a blood meal containing DENV-2 and JNJ-A07 (100 µM). The presence of JNJ-A07 markedly reduced the number of infected mosquito bodies at day 3 post-exposure, compared to the control group (11% vs 78%). Furthermore, no virus dissemination to mosquito secondary organs was observed at day 7, in contrast to the control group (0% vs 50%). This research warrants further investigation. Our results suggest that mosquito exposure to a potent antiviral drug in the blood of treated humans could significantly decrease DENV transmission by mosquitoes to other humans and therefore might impact the magnitude of DENV outbreaks.

Acknowledgments. This work received funding from the Flanders Agency Innovation & Entrepreneurship (VLAIO O&O grant HBC.2019.2906).

033. AT-752, A Double Prodrug of a Guanosine Nucleotide Analog, is Effective Against Yellow Fever Virus in a Hamster Model.

Abbie Weight, B.S.[1], Kai Lin, Ph.D.[2], Steven Good, M.S.[2], Adel Moussa, Ph.D.[2], Jean-Pierre Sommadossi, Ph.D.[2], Justin Julander, Ph.D.[1]

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Yellow fever virus (YFV), a re-emerging mosquito-borne flavivirus, causes significant morbidity and mortality in Africa and the Americas. An effective vaccine is available, yet there are no antivirals available to treat the disease. AT-752 is an orally available double prodrug of a guanosine nucleotide analog. Previous research showed that the free base of AT-752 effectively inhibits YFV in vitro, with a 90% effective concentration (EC_{90}) of 0.15 µM. We evaluated the in vivo efficacy of AT-752 against YFV in a hamster model. Administration of 1000 mg/kg/d AT-752, initiated 4 hours pre-infection or 2 days post infection (dpi) and continuing for 7 days, significantly improved survival, viremia, and serum alanine aminotransferase (ALT) levels in YFV-infected hamsters. AT-752 treatment initiated 4 hours before viral challenge resulted in significantly reduced viremia and ALT to levels similar to those of sham-infected animals. Treatment initiated 2 dpi resulted in a >2$\log_{10}$-fold decrease in viremia and a 53% decrease in serum ALT ($p<0.001$ for both) when compared with placebo controls. AT-752 treatment significantly improved survival regardless of dose or time of treatment initiation. Finally, a pharmacokinetic study in hamster tissues found the highest levels of the active metabolite in the kidney and liver, the organs most affected by YFV. These results show that AT-752 has efficacy in vivo against YFV and thus justify further research into the clinical potential of this compound as a treatment for YFV.

[This work was supported in part by the NIAID, NIH contracts 75N93019D00021, Task B01 and HHSN272201700041I, Task A11]

034. Guanine Quadruplexes in the RNA Genome of the Tick-borne Encephalitis Virus: A New Antiviral Target

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Guanine quadruplexes (G4s) are unique non-canonical secondary structures of nucleic acids, playing critical roles as important regulatory elements of viral replication. In our study, we identified seven putative G4s in the genome of the tick-borne encephalitis virus (TBEV), a causative agent of tick-borne encephalitis, whis is one of the most serious neuroinfection in European and Asian continent. Six of the identified G4s were located in the TBEV genes NS1, NS4b and NS5. The G4 denoted as TBEV-5 was located at NS4b/NS5 border and was found to be conserved among all known flaviviruses. Only one G4 was found in the 3'-untranslated region. The formation of parallel stranded G4s was confirmed using a panel of biophysical methods, such as circular dichroism and absorption spectroscopy analyses of synthetic oligonucleotides derived from the predicted TBEV G4 sequences. TBEV-5 and its non-G4 mutated variant were inspected for their interactions with small-molecule G4-binding ligands in vitro. Pyridostatin, carboxy-pyridostatin, N-methylmesoporphyrin IX, TMPyP4, PhenDC3, and berberine effectively inhibited both TBEV replication in cell-based systems and in vitro RNA synthesis by the flaviviral RNA-dependent RNA polymerase. Moreover, the G4-specific TBEV mutant with highly stabilized TBEV-5 quadruplex exerted increased sensitivity to G4-binding ligands, strongly decreased replication fitness and significantly altered plaque morphology compared with wild-type. The data indicate that G4s are important for efficient TBEV replication and represent suitable new target to combat the TBEV infection.

035. In Situ Click Chemistry Applied to Bunyavirales: From Conventional Drug Design to Enzymes Assembling Their Own Inhibitors like LEGOs®

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With a worldwide repartition and limited therapeutic options reported, neglected *Bunyavirales* viruses represent a major public health issue. The replication machinery of these viruses is governed by the intricate L-protein that displays RNA-dependent RNA-polymerase activity (RdRp) and endonuclease (EndoN) activity in its N-terminal end.[1,2] This key protein, responsible for the cap-snatching mechanism that allows the viral transcription, was identified as a promising target to develop pan-genus antivirals. Its catalytic mechanism of RNA hydrolysis mediated by Mg^{2+} ions enables the development of diketo-acids (DKAs) metal-chelating inhibitors.[3] Recently, rational drug-design concepts have made their way with the emergence of Target-Guided-Synthesis (TGS), a powerful method that directly involves the target that assembles its own inhibitors In Situ like LEGOs®. Herein, we describe the use of *Bunyavirales* EndoN active sites as reaction vessels for the *In Situ* generation of their own highly specific metal-chelating inhibitors. DKA anchor molecules bearing an azide moiety were synthesized using an optimized pathway and EndoNs affinity towards them was assessed using TSA and MST. The *In Situ* Click Chemistry experiment was designed in 96-well plates using biochemical conditions and various combinations of DKA-azide and alkyne fragments to produce 1,4-triazolyl-diketo-acids (NT-DKAs) in the presence of the enzyme. HIT molecules resulting from this fragment-based screening are identified by HPLC-MS and are synthesized on large scale to enable their full biological characterization.

1. Reguera et al., *PLoS* Pathog., 2010, 6(9), e10011011

2. Morin et al., *PLoS* Pathog., 2010, 6(9), e1001038

3. Saez-Ayala et al., *IUCrJ*, 2018, 5(2), 223-235

036. The COVID-19 Pandemic: A View from the Bench

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All known recently emerged human coronaviruses likely originated in bats. We used a single experimental platform based on human lung-only mice (LoM) to demonstrate efficient in vivo replication of all recently emerged human coronaviruses (SARS-CoV, MERS-CoV, SARS-CoV-2) and two highly relevant endogenous pre-pandemic SARS-like bat coronaviruses. Virus replication in this model occurs in bona fide human lung tissue and does not require any type of adaptation of the virus or the host. Our results indicate that bats harbor endogenous coronaviruses capable of direct

transmission into humans. Further detailed analysis of pandemic SARS-CoV-2 in vivo infection of LoM human lung tissue showed predominant infection of human lung epithelial cells, including type II pneumocytes present in alveoli and ciliated airway cells. Acute SARS-CoV-2 infection was highly cytopathic and induced a robust and sustained Type I interferon and inflammatory cytokine/chemokine response. Finally, we evaluated a therapeutic and pre-exposure prophylaxis strategy for coronavirus infection. Our results show that therapeutic and prophylactic administration of EIDD-2801, an oral broad-spectrum antiviral, dramatically inhibited SARS-CoV-2 replication in vivo and thus demonstrated significant potential for the prevention and treatment of COVID-19. Finally, EIDD-2801 treatment reduced the long-term sequela associated with virus infection further highlighting the benefits of early treatment of infection.

037. Pyrimidine Inhibitors Synergize with Nucleoside Analogues to Block SARS-CoV-2

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The SARS-CoV-2 pandemic continues to cause widespread infections. While vaccines have been rapidly developed to combat SARS-CoV-2, there has been a dearth of antiviral therapeutics. There is an urgent need for therapeutics which has been amplified by the emerging threats of variants that may evade vaccines. Large scale efforts are underway to identify antiviral drugs and we screened ~18,000 drugs for antiviral activity using live virus infection in human respiratory cells and validate 122 drugs with antiviral activity and selectivity against SARS-CoV-2. Amongst these candidates are nucleoside analogs, the largest category of clinically used antivirals including the approved remdesivir and molnupiravir. RNA viruses rely on a high supply of nucleoside triphosphates from the host to efficiently replicate, and we identified a panel of host nucleoside biosynthesis inhibitors as antiviral. Moreover, we found that combining pyrimidine biosynthesis inhibitors with antiviral nucleoside analogs synergistically inhibits SARS-CoV-2 infection in vitro and in vivo against emerging strains of SARS-CoV-2 suggesting a clinical path forward for combinations.

038V. Mechanochemistry and Drug Targeting of the SARS-CoV-2 Replication-transcription Complex from a Single Molecule Perspective

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The SARS-CoV-2 pandemic has been and still is a terrible burden for our societies. While the vaccine response has been incredibly fast, drugs development is still in its infancy, and lack to cure patients infected by SARS-CoV-2, or by future coronavirus outbreaks. The main antiviral drug target is the replication-transcription complex (RTC), a conserved large multiprotein machinery in charge of replicating and transcribing the ~30 kb long positive single stranded (ss) RNA coronavirus genome. The RTC is made of a core RTC formed of the RNA polymerase nsp12, and the associated factors nsp7 and nsp8, in a stoichiometry 1:1:2, to which associates many factors. While the cryoEM revolution has revealed the structure of many partial RTCs, functional studies have lagged behind, shadowing our knowledge on the structure-function relationship of these RTCs, and delaying the finding drug targets and novel antiviral drugs. To fill this gap, we have pioneered a high throughput magnetic tweezers assay to monitor the elongation dynamics of the SARS-CoV-2 RTC over 1 kilobase long template at near single base resolution in the presence of all NTPs. Here, I will present how we applied this assay to reveal the nucleotide addition cycle of SARS-CoV-2 core RTC (Bera, Seifert et al., Cell Reports 2021), and the mechanism of action of several nucleotide analogs, such as Remdesivir and ddhC (Seifert, Bera et al., eLife 2021). I will also present our latest investigations of the core RTC mechanochemistry when including other viral co-factors.

039. Profile of PBI-0451 an Orally Administered 3CL Protease Inhibitor of SARS-CoV-2 for COVID-19

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There is a need for orally self-administered direct-acting antivirals (DAA) to inhibit SARS-coronavirus 2 (CoV2) viral replication to reduce the risk of developing severe disease. The main protease of CoV2 represents an attractive druggable target. It is essential for processing of the viral polyprotein to liberate the components needed to assemble a functional viral replicase required for the production more virions in the spread of the infection. Computational analyses of the 3CL active site topography and variations across the known human pathogenic coronaviridae allowed

the identification of key conserved ligand interactions, and the creation of a consensus binding pocket for the design of reversible-covalent inhibitors with pan-coronaviral activity. Here, we report the design, discovery, and characterization of PBI-0451, a selective, orally bioavailable small molecule. PBI-0451 has potent antiviral activity against SARS-CoV-2 in cell-based assays and is also active against main proteases from SARS, 229E, HKU1, OC43, NL63, and MERS. It inhibits replication of of all SARS-CoV-2 variants evaluated without cytotoxicity in cell-based assays. Resistance to PBI-0451 through serial passaging in cell culture has been difficult to attain. Screens for off-target activity on host cysteine proteases and other targets revealed no significant concerns. PBI-0451 demonstrated oral bioavailability in all species tested, without relevant adverse findings in GLP toxicology studies, supporting its entry into clinical development. In an ongoing Phase I clinical trial in healthy human participants, PBI-0451 has been well-tolerated and has demonstrated favorable pharmacokinetics supporting its potential as a single oral agent for the treatment and prevention of COVID-19.

040. Strategies to Interfere with Nucleotide Excision by the 3′-to-5′ Exoribonuclease from SARS-CoV-2

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Effective antiviral therapeutics are needed to treat SARS-CoV-2 infections. While some of the most efficacious antivirals are nucleos(t)ide analogs, a coronavirus-specific complication is the virus-encoded 3′-to-5′ exoribonuclease (ExoN) which is capable of excising antiviral nucleotides. ExoN, composed of a complex of nsp14 and nsp10, is critical to viral replication as genetic inactivation of nsp14 leads to decreased replication fidelity and increased sensitivity to mutagenic nucleoside analogs. These findings underscore the need to understand ExoN activity and the potential to antagonize ExoN proofreading to more effectively address SARS-CoV-2 infection with antiviral nucleotides. We have expressed and purified components of ExoN: nsp14 and nsp10. We have established a robust, quantitative system to study the specificity and efficiency of ExoN activity and elucidate the kinetic and chemical mechanism of proofreading. We find that a dsRNA substrate, resembling a primed template, is preferred over ssRNA. ExoN appears to be highly processive under conditions of enzyme excess. However, consistent with the enzyme contributing to proofreading, ExoN is actually quite distributive, hydrolyzing only one to two nucleotides in a single binding event. We find no difference in the excision of paired vs mispaired ends, suggesting that access may initiate proofreading instead of mispair recognition. Finally, we have discovered modifications to the 3′-RNA terminus which antagonize or completely block ExoN-catalyzed excision. We conclude that design of ExoN-resistant, antiviral nucleotides will be feasible.

041. Efficacy in a SARS-CoV-2 African Green Monkey Model Validates a Prodrug Approach for Oral Delivery of Remdesivir Parent Nucleoside GS-441524

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Remdesivir (RDV, VEKLURY®) exhibits clinical efficacy in hospitalized patients and out-patient settings and is currently the only FDA-approved direct-acting antiviral treatment for SARS-CoV-2 infection. GS-441524, the RDV parent nucleoside, and GS-621763, a tri-ester prodrug of GS-441524 are both active against SARS-CoV-2, albeit with lower in vitro potencies than RDV. Unlike GS-441524, the prodrug GS-621763 shows high oral bioavailability across multiple species and can deliver systemic levels of GS-441524 sufficient for potent efficacy in ferret and mouse SARS-CoV-2 models. To further characterize in vivo antiviral efficacy from systemic GS-441524, we evaluated GS-441524 and GS-621763 treatments in the SARS-CoV-2 African green monkey (AGM) model. Target GS-441524 plasma concentrations required intravenous (IV) infusion of GS-441524 due to its low (<10%) AGM oral bioavailability, while GS-621763 achieved target plasma exposures following oral dosing. To assess dose responses, AGMs were treated with either 7.5 or 20 mg/kg IV GS-441524 or 60 or 120 mg/kg oral GS-621763 starting ~8h post-infection, and daily thereafter for 5 days, and compared to AGMs treated with matching vehicle controls. Following GS-441524 treatment, dose-dependent reductions in SARS-CoV-2 RNA and infectious viral loads were observed in bronchioalveolar lavage fluid (BALF) over time and in terminal respiratory tract tissues. Both doses of GS-621763 reduced SARS-CoV-2 RNA and infectious virus in BALF. These results indicate that SARS-CoV-2 AGM antiviral efficacy can be achieved with sufficient circulating levels of GS-441524. However, due to the variable preclinical oral bioavailability of GS-441524 itself, orally bioavailable prodrugs of GS-441524 are being pursued as potential therapies for COVID-19.

042. A Dual Mechanism of Action of AT-527 Against SARS-CoV-2 Polymerase

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The ongoing COVID-19 pandemic, caused by SARS-CoV-2, has emphasized the urgent need for antiviral therapeutics. The viral RNA-dependent-RNA-polymerase (RdRp, nsp12) is a promising target, with polymerase inhibitors successfully used for the treatment of several viral diseases. We have previously demonstrated that the SARS-CoV RdRp complex is at least 10-fold more active than any other viral RdRp known. It possesses both unusually high nucleotide incorporation rates and high-error rates, allowing facile insertion of various nucleoside analogues into the RNA during replication. The guanosine analogue AT-527 (and its active triphosphate form AT-9010) represents a promising candidate, and recently entered phase III clinical trials for the treatment of COVID-19. Here we report a 2.98 Å cryo-EM structure of the SARS-CoV-2 nsp12-nsp7-nsp8$_2$-RNA complex, showing AT-9010 bound at three sites of nsp12. In the RdRp active-site, one AT-9010 is incorporated at the 3′ end of the RNA product strand. Its modified ribose group (2′-fluoro, 2′-methyl) prevents correct alignment of the incoming NTP, in this case a second AT-9010, causing immediate termination of RNA synthesis. The third AT-9010 is bound to the N-terminal domain of nsp12 - known as the NiRAN. In contrast to native NTPs, AT-9010 is in a flipped orientation in the active-site, with its guanine base unexpectedly occupying a previously overlooked cavity. AT-9010 outcompetes all native nucleotides for binding, inhibiting NiRAN nucleotide-transferase activity. Our results suggest a dual mechanism of action of AT-527 in the targeting of both RdRp and NiRAN activites, in line with a therapeutic use for COVID-19.

043. Dual Inhibition of SARS-CoV-2 and Human Rhinovirus with Protease Inhibitors in Clinical Development

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The 3-chymotrypsin-like cysteine protease (3CLpro) of SARS-CoV-2 is considered a major target for the discovery of direct antiviral agents. We previously reported the evaluation of SARS-CoV-2 3CLpro inhibitors in a novel self-assembled monolayer desorption ionization mass spectrometry enzymatic assay. The assay was further improved by adding the rhinovirus HRV3C protease to the same well as the SARS-CoV-2 3CLpro. High substrate specificity for each enzyme allowed the proteases to be combined in a single assay reaction without interfering with their individual activities. This novel duplex assay was used to profile a diverse set of reference protease inhibitors. The protease inhibitors were grouped into three categories based on their relative potency against 3CLpro and HRV3C including those that are: equipotent against 3CLpro and HRV3C (GC376 and calpain inhibitor II), selective for 3CLpro (PF-00835231, PF-07321332/PAXLOVID™, calpain inhibitor XII, boceprevir), and selective for HRV3C (rupintrivir). Structural analysis showed that the combination of minimal interactions, conformational flexibility, and limited bulk allows GC376 and calpain inhibitor II to potently inhibit both enzymes. In contrast, bulkier compounds interacting more tightly with pockets P2, P3, and P4 due to optimization for a specific target display a more selective inhibition profile. Consistently, the most selective viral protease inhibitors were relatively weak inhibitors of human cathepsin L. The assay was further optimized to measure IC_{50} values in the picomolar range for tight binding inhibitors. Taken together, these results can guide the design of cysteine protease inhibitors that are either virus-specific or retain a broad antiviral spectrum against coronaviruses and rhinoviruses.

044. In Vitro Selection and Characterization of a SARS-CoV-2 Isolate Resistant to Remdesivir

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As new SARS-CoV-2 antivirals are being developed it becomes critical to learn if and how the virus develops resistance. To our knowledge there has not been any evidence of remdesivir resistance arising in patients. In this work we investigated if resistance against remdesivir can be selected *in vitro*.

A SARS-CoV-2 isolate, obtained from a Belgian patient in February 2020 and closely related to the prototypic Wuhan-Hu-1 2019-nCoV (Wuhan isolate), was passaged on VeroE6 cells in the presence GS-441524, the parent nucleoside of remdesivir, starting just below the EC_{50} (1 μM). The concentration of compound could be increased gradually to 20 μM by passage 14 (day 45) and virus was further replicated at this concentration until passage 21. Genetic analysis of this last passage showed mutations in the polymerase gene that correlate with amino acid changes S759A + A777S and phenotypic analysis showed that the EC_{50} of GS-441524 was increased more than 10x (EC_{50}>50 μM). Interestingly S759A is located immediately next to the amino acids D760 and D761 that are part of the aspartic acid triad at the active site. Confirmation using reverse genetics, analysis of the replication capacity of the virus and potential cross-resistance with other antivirals is ongoing. For this experiment it took a significant amount of time (> 10 passages) before we could increase the compound concentration 10x. This is a first indication of a significant barrier to resistance development and thus a low possibility of resistance selection during a short clinical treatment (~5 days).

045. Oral Inhibitors of the SARS-CoV-2 Main Protease for the Treatment of COVID-19

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Small molecule inhibition of the viral main protease (Mpro) has been a successful anti-viral therapeutic strategy in HIV and HCV. Structural insight on the SARS-CoV-2 Mpro and previous small molecule experience with intravenous SARS-CoV-1 inhibitors gave a starting point for an oral Mpro inhibitor program in response to the COVID-19 outbreak. The discovery of PF-7321332, the first oral SARS-CoV-2 Mpro inhibitor to reach clinical development, will be described.

046. Picomolar Covalent Reversible Inhibitors of CoVs Main Proteases Effectively Inhibit SARS-CoV-2 Replication: Design, Synthesis, Biological Evaluation, and X-Ray Structural Characterization

Rolando Cannalire, Ph.D.[1], Francesca Esposito, Ph.D.[2], Irina Stefanelli, M.S.[1], Angela Corona, Ph.D.[2], Francesco Di Leva, Ph.D.[1], Emilia Cassese, M.S.[1], Paola Storici, Ph.D.[3], Elisa Costanzi, Ph.D.[3], Enzo Tramontano, Ph.D.[2], **Vincenzo Summa, Ph.D.[1]**
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The catastrophic SARS-CoV-2 outbreak has resulted in the pandemic COVID-19. Although in industrialized countries restrictive measures and vaccinations are limiting the pandemic,[1] the only FDA authorized antiviral is the repurposed iv drug Remdesivir. Two oral candidates, the nucleoside Molnupiravir and Paxlovid (PF-07321332), a peptidomimetic inhibitor of the Main protease (M^{pro}), showed respectively 50% and 89% efficacy in Phase2/3, strongly support their possible FDA Emergency Use Authorization. Considering the limited therapeutic options, new antivirals are needed for people unable to receive vaccinations in developing countries or to mitigate the harmful consequences of the new infections, and to fight emerging variants. M^{pro} is a cysteine protease with an almost unique P2–P1 specificity for Leu-Gln, highly conserved across CoVs.[3]Compounds targeting the M^{pro} of different CoVs were repurposed against SARS-CoV-2 and some new structurally close peptidomimetics acting as covalent reversible inhibitors have been reported, but still, there is a room for further development.[2]In this context, we report on a new series of tripeptides as covalent reversible inhibitors of SARS-CoV-2 M^{pro}, mainly investigating the effect of differently functionalized proline residues in the P2 position. The most potent compounds showed pM potency against the M^{pro} of SARS-CoV-2 and MERS-CoV and were able to inhibit viral replication in the nM potency without detectable cell toxicity. Herein, we will describe

the rational design, molecular modelling, synthesis, biological evaluation in biochemical, biophysical and phenotypic assays, and X-ray crystallography around these new inhibitors.

Izsa, V. et al. Clin Immunol. 2021,222,108634

Cannalire, R. et al. J. Med. Chem. 2020, doi.org/10.1021/acs.jmedchem.0c01140

047V. Clinical Update on Viral RNA Targeting Agents for Chronic Hepatitis B

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In patients with chronic HBV infection, persistent high antigen load is a major factor driving the exhaustion of antiviral adaptive immunity. Currently, NUC administration induces virema suppression but not viral antigen reduction. HBV RNA targeting agents aiming at reducing antigen burden represent a promising class of drugs that could be combined with direct immunotherapies in the hope of achieving a synergistic effect. Development of transcription inhibitors and and mRNA destabilizers has been hampered by toxicities inherent with targeting a host pathway. In contrast, both the Gal-Nac conjugated siRNAs and the naked ASOs, administered subcutaneously, appear to be safe and well tolerated. All siRNAs in Phase II clinical development (JNJ-3989; VIR-2218; RG-6346; AB-729) achieve on-treatment HBsAg responses, which is durable for several months after the final dose. Unfortunately, the results also showed a plateauing of HBsAg and no patients achieved HBsAg loss. Interestingly, the first study combining an siRNA with pegylated IFN showed a synergistic effect on HBsAg reduction associated with ALT elevations. In contrast, biweekly GSK-836 (ASO) 300mg dosing achieved 3-4 log reductions in HBsAg within 28 days, associated with ALT elevation that may suggest some level of immune restoration. Whether this will lead to sustained HBsAg loss and functional cure is currently investigated in a phase II trial. Results in mouse models showed that siRNA knockdown of HBV transcripts followed by therapeutic vaccination allowed viral clearance. Ongoing phase II clinical trials will determine if functional cure can be achieved with this type of combination therapy.

048. Discovery and Development of HBV Core Inhibitors for the Treatment of Chronic Hepatitis B Infection

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Core inhibitors are a new class of antivirals with the potential to deepen viral suppression and improve cure rates in patients with chronic HBV infection when used in combination regimens. Core inhibitors interact allosterically with HBV core protein and modulate assembly and stability of nucleocapsids. Core protein is critical both early and late in HBV replication, and thus core inhibitors have multiple MOAs including: (1) inhibition of pgRNA encapsidation (preventing assembly and release of new viral particles); (2) blocking intracellular amplification of cccDNA; and (3) disrupting incoming capsids, which prevents de novo cccDNA formation. We believe potent activity against all three MOAs is likely to be important for maximal viral suppression. Multiple chemically-diverse core inhibitors are progressing through preclinical, phase 1, and phase 2 development. Phase 1 studies have confirmed that core inhibitors have potent antiviral activity including multilog suppression of both serum HBV DNA and RNA, with the latter effect not observed with nucleos(t)ide reverse transcriptase inhibitors (NrtIs). Phase 2 combination studies have demonstrated that addition of a core inhibitor to NrtIs deepens suppression of both HBV DNA and RNA compared to NrtIs alone. Importantly, there has been no evidence for emergence of core inhibitor resistance in patients compliant with combination therapy. Current studies are investigating the curative potential of triple combination regimens consisting of core inhibitors, NrtIs, and various third agents (siRNA, interferon, TLR7 agonist). As these agents are further evaluated, next-generation core inhibitors with significantly greater potency, particularly against cccDNA formation, are in early clinical development.

049. Explore Viral Infection to Probe Roles of Protein Deamidation

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Herpesviruses are ubiquitous human pathogens that cause significant morbidity and mortality, particularly in immune-compromised patients. Despite of active immune response, herpesviruses establish persistent infection in diverse tissues. Studying viral immune evasion, we discovered that herpesviruses deploy pseudo-enzymes or bona fide enzymes to target key pattern recognition receptors for deamidation and evasion. Interestingly, these studies implicate cellular glutamine amidotransferases in deamidating proteins. Glutamine amidotransferases catalyze the synthesis

of nucleotides, amino acids, glycoproteins and NAD, which constitute building blocks of cell proliferation and viral replication. Exploring viral infection and host defense system, we probed the roles of glutamine amidotransferases and protein deamidation in fundamental biological processes. In one study, we report that protein deamidation shunts RelA from mediating inflammatory response to aerobic glycolysis to fuel cell proliferation. As such, cancer cells exploit RelA deamidation to promote glycolysis and cell proliferation. In another study, we discovered that SARS-CoV-2 activates CTP synthetase 1 (CTPS1), a rate-limiting enzyme of CTP synthesis, to fuel nucleotide synthesis. Surprisingly, activated CTPS1 deamidates IRF3 to mute interferon induction, thereby evading host innate immune defense. Pharmacological inhibition of CTPS1 not only depletes the nucleotide pool, but also restores antiviral interferon response, thus impeding SARS-CoV-2 replication. These findings uncover pivotal roles of protein deamidation and metabolic glutamine amidotransferases in cell proliferation and immune defense, establishing crosstalk between otherwise disconnected processes.

050. LAVR-289, a New Broadly Active Acyclonucleoside Phosphonate Prodrug is Highly Effective in the SCID-Hu Mouse Model of Varicella Zoster Virus Replication

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VZV causes varicella (chicken pox) and establishes lifelong latency in neurons. The virus may reactivate years later as herpes zoster (shingles). These infections are characterized by vesicular skin lesions. Herein, we report on a novel acyclonucleoside phosphonate prodrug, LAVR-289, a uridine analog. In cell-based assays, LAVR-289 has potent antiviral activity against multiple DNA viruses, including VZV, HCMV and poxvirus. In ARPE-19 cells, LAVR-289 was highly potent against VZV-BAC-Luc (wild type) and the acyclovir-resistant variant, VZV-BAC-Luc-ACV^R, with an approximate EC_{50} of 0.01 μM (10 nM). Notably, it was nearly 100-times more potent than the approved drugs acyclovir and cidofovir. In human skin organ culture, LAVR-289 prevented VZV spread at >1 μM. In SCID-Hu mice with skin xenografts (N=12 mice per group), LAVR-289 prevented VZV spread when administered once daily by the subcutaneous route on days 3-9 post infection. Doses of 26, 13, and 6.5 mg/kg were significantly effective (p=0.029). When administered every other day, 26 and 13 mg/kg remained effective (p=0.0018). LAVR-289 was well tolerated and did not cause weight loss or signs of distress. LAVR-289 is a promising compound with potential for development as a novel drug against VZV infections. This project was supported by Région Centre Val de Loire, FérI2 and DGA/AID (Deanalpovir) and by the NIAID DMID contract HHSN272201700030I.

052. Characterization of the N-hydroxypyridinediones (HPD) and the N-hydroxynapthyridinones (HNO) as HBV RNase H inhibitors

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N-hydroxypyridinediones (HPD) and *N*-hydroxynapthyridinones (HNO) are compound classes that inhibit metalloenzymes. They are effective against the Hepatitis B Virus (HBV) ribonuclease H, suppressing HBV replication at sub-micromolar concentrations. Previously, the best compounds from these classes had selectivity indexes of ~350 (HPD) and ~100 (HNO). We are characterizing the efficacy, cytotoxicity, pharmacological properties, and off-target effects of newly synthesized HPDs and HNOs. Cytotoxicity was assessed using MTS (mitochondrial function) in hepatic cell lines and primary human hepatocytes. At physiologically relevant pHs, we mimicked passive diffusion across the epithelium using parallel artificial membrane permeability assays (PAMPA), active transport and efflux using Caco-2 cells, and solubility. Phase I and II metabolism was determined by calculating compound half-life in microsome and hepatocyte stability assays, respectively. HPD and HNO toxicities were similar in the hepatic cell lines and all compounds tested had CC_{50}s >100 μM in PHHs. Additionally, compounds in both compound classes had $t_{1/2}$ > 4 hr. However, the HPDs were more soluble and passively permeable at all pHs, and the HPDs were more effective at suppressing plus polarity strand synthesis – two had EC_{50} values of 11 and 65 nM, resulting in selectivity indexes of 500 and 1500. The best HNO had an SI of 200. These results advance HPD and HNOs as HBV RNase H inhibitors, and indicate that the HPDs are a promising chemotype while HNOs may require more extensive chemical modification.

053. Resistance Analysis in a Phase 2 Clinical Trial with the Helicase-Primase Inhibitor Pritelivir in Immunocompromised Adults with Acyclovir Resistant Herpes Simplex Virus (HSV) Infection

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Pritelivir is an oral anti-HSV drug targeting the helicase-primase. Safety and efficacy against acyclovir resistant mucocutaneous HSV infections were investigated in immunocompromised adults in a Phase 2 trial. In Part A, pritelivir treatment was compared to foscarnet. In Part B, patients were enrolled who were acyclovir resistant and foscarnet resistant or intolerant, a population with only limited treatment options. Resistance characterization was performed when lesions did not heal at the end of treatment or new lesions were present at follow-up. Lesion swabs were used for genotypic analysis by Sanger sequencing. When necessary, next generation sequencing was conducted. In case of inconclusive results, phenotypic testing was performed. In Part A, resistance to pritelivir was detected in 1 of 15 pritelivir-treated subjects (6.7%) and resistance to foscarnet in 1 of 7 foscarnet-treated subjects (14.3%). No resistance to pritelivir was identified in 8 subjects in Part B. In all, 1 of 23 (4.3%) subjects developed pritelivir resistance. The subject with resistance to pritelivir had a treatment-emergent mutation at a position in the UL5 helicase known for mediating resistance from *in vitro* studies. Resistance was confirmed by phenotypic testing. The resistance to foscarnet was mediated by a known mutation in the UL30 polymerase. This is the first description of pritelivir resistance emerging in the clinic. The mutation mapped at a position associated with reduced pritelivir susceptibility based on cell culture data. Overall, the incidence of HSV resistance among subjects on pritelivir appeared to be lower than on foscarnet.

060. Intermittent Therapy with Helicase-Primase Drug Candidate IM-250 Reduces Reactivation Competency of Latent Neural Herpes Simplex Virus Infections

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Herpes simplex virus (HSV) causes widespread genital and oropharyngeal disease; less often, encephalitis, sight impairing keratitis, and neonatal or disseminated herpes. HSV establishes lifelong latent infections in neurons supporting viral reactivation and recurrent disease. Current treatments include valacyclovir, acyclovir or famciclovir. More effective therapies are needed for CNS infections, resistant viruses, and ideally for silencing recurrences after cessation of treatment. Recently, we published the profile of IM-250 with potent in-vitro and in-vivo activity against HSV including nucleoside-resistant HSV due to its different mechanism of action. Early therapy of genital herpes in guinea pigs significantly reduces primary disease, ganglionic viral load, and recurrent disease. Importantly, IM-250 reduced recurrences for a time after cessation of treatment. To further investigate the ability of IM-250 to influence reactivation after latency has been established and after cessation of treatment, viral reactivation at the single neuron level was examined in the mouse ocular model. Beginning 45 days after HSV-1 ocular infection, 4 cycles of intermittent treatment (IT, 1-week IM-250 or placebo in food followed by 2-weeks normal chow) were initiated. Viral reactivation induced 15 days after final IT resulted in 2-fold fewer positive ganglia (7/24 and 14/24), and a 3.6-fold reduction in total neurons undergoing reactivation in IM-250 vs placebo treated mice (p=0.002). Confirmed in a smaller study, these findings support the hypothesis that intermittent treatment with IM-250 alters the pool of latent reactivatable virus, a novel and a major advance for HSV therapies. Clinical trials to confirm these murine findings are planned.

061. A Bifunctional Immune Modulator Exhibits Potent Antiviral Activity in HBV Infection Models

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Type-I interferons (IFN-I) have shown limited ability to cure patients with chronic hepatitis B (CHB), thus more effective treatments are needed to achieve cure in a significant proportion of patients. Previously, we demonstrated that the simultaneous stimulation of CD40 and IFN-I pathways in vitro and in vivo HBV infection models increased the antiviral efficacy compared to IFN-I alone. The combination of IFN-I and CD40L, on primary human hepatocytes (PHH) and in AAV/HBV-infected mice, showed a significant increase in anti-HBV activity when compared to either CD40L or IFN-α alone. Fusion of IFN-I molecules to an anti-CD40 agonistic mAb yielded a bifunctional molecule active on both CD40 and IFNR reporter cells capable of delivering both activities to HBV infected hepatocytes in vivo. The fusion molecule is able to reduce viral products from HBV-infected PHH treated for a period of 4 days after infection at picomolar concentrations without cytotoxicity. Stimulation of CXCL10 release and anti-HBV activity in infected PHH treated for 1 day followed by washout period of 3 days was maintained. These results demonstrate the feasibility of combined stimulation of CD40 and IFN-I pathways with a single bifunctional molecule to achieve potent activity against HBV.

062. The Nucleoside Analog Antiviral CMX521 Inhibits SARS-CoV-2 in Human Airway Epithelial Cell Cultures and Exhibits Prophylactic and Therapeutic Efficacy Against Respiratory Disease in a Mouse Model of SARS-CoV-2 Infection

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The SARS-CoV-2 pandemic necessitates continued development of novel therapies with improved activity/resistance profiles. CMX521, a ribonucleoside analog, inhibits SARS-CoV-2 replication in primary human airway epithelial cell cultures (average EC50=0.9μM). CMX521 is not genotoxic or mitotoxic, has a favorable toxicology profile (rats/dogs, oral GLP), and was generally well-tolerated up to 2400mg in a healthy volunteer Phase 1 study of oral CMX521. In vivo evaluation of aerosol CMX521 was performed in uninfected BALB/c mice (tolerability/PK) or mice infected intranasally with 10,000 PFU of SARS-CoV-2-MA10 (N=79). Prophylactic administration of CMX521 (q8 hours) starting 24 hours prior to infection reduced average viral titers in lung on day 4 post-infection by 3.62 log10 and prevented weight loss/clinical progression versus placebo (n=9/group). The most comprehensive single study randomized mice to CMX521 at 0, +8 or +16 hours post-infection or placebo (n=6/group). CMX521 treatment significantly reduced lung viral RNA (Kruskal-Wallis $p<0.0001$) and viral titer ($p<0.0001$) at day 4 post-infection relative to placebo. CMX521 treatment post-infection also protected mice from clinical symptoms of disease, significantly reducing weight loss and decreasing lung pathology compared to placebo ($p<0.0001$). For example, CMX521 initiated 16 hours post-infection reduced average viral lung titer by 2.56 log10, prevented weight loss and reduced average clinical score from 3 to 0 (day 4). Overall, no gross safety or tolerability issues were identified in CMX521 treated mice. These data indicate that aerosol CMX521 is an effective prophylactic and therapeutic for SARS-CoV-2 in a preclinical animal model and support further development as an antiviral against coronaviruses.

063. Structural and Mechanistic Characterization of Non-Neutralizing Antibodies Targeting Crimean-Congo Hemorrhagic Fever

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Crimean-Congo Hemorrhagic Fever Virus (CCHFV) causes a debilitating hemorrhagic fever with a mortality rate as high as 40%. With a 2017 outbreak in Spain illustrating CCHFV's continued ability to expand its endemic area and no approved vaccine or therapeutics available, CCHFV is viewed as a priority public health threat by the WHO. Recently, the non-neutralizing monoclonal antibody (mAb) 13G8 has been shown to target the CCHFV glycoprotein GP38 and protect against lethality in a CCHFV mouse model with diverse strains. Here we biochemically reveal how strain-strain differences among GP38s affect interactions with 13G8 as well as a new mAb CC5-C17. The latter was identified among five mAbs derived from the blood of recovered CCHFV patients targeting CCHFV GP38 and exhibits superior binding affinity for GP38 over 13G8. To better understand the molecular origins of this phenomena, X-ray crystallography structures of GP38 from a human clinical isolate CCHFV strain, as well as it in complex with 13G8 and CC5-C17, were obtained. This structural information not only identified what GP38 regions can serve as therapeutically relevant epitopes for protection against CCHFV, but also provides a molecular basis to predict a wide swath of CCHFV strains that would be susceptible to monoclonal treatment using 13G8 or CC5-C17. This information coupled with of in vivo efficacy data paves the way for an effective future monoclonal antibody therapeutic towards CCHFV.

100. Zebrafish larvae as in vivo model for Rift Valley fever virus replication and pathology

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Rift Valley fever virus (RVFV) is a mosquito-borne pathogen able to infect ruminants and humans. It is on the WHO list of ten priority diseases, partly because a subset of patients develops severe symptoms like permanent blindness, encephalitis and hemorrhagic fever. To understand the mechanisms of RVFV disease and acquire *in vivo* data on antivirals earlier and easier in a high biosafety setting we have established a new *in vivo* model using zebrafish (*Danio rerio*) larvae. After infection viral RNA levels reach as high as ~4 $\log_{10}$ copies per larva with RVFV mostly observed in the liver, sensory nervous system and vascular system of the larvae. Replication of the virus was completely inhibited by 2'-Fluoro-2'-deoxycytidine, a polymerase targeting antiviral know to inhibit RVFV, by simply adding it to the swimming water. To further characterize this infection model we are currently characterizing the innate immune response and roles of stat1a and stat1b in RVFV infection. To that end, we have created Tg:stat1a$^{-/-}$ and Tg:stat1b$^{-/-}$ zebrafish lines. Disrupting the JAK/Stat pathway by either administering ruxolitinib or using Tg:stat1a$^{-/-}$ larvae increases RNA levels up to ~6 $\log_{10}$ copies per larva. Remarkably, larvae lacking stat1b show much lower levels of viral replication. Since stat1b is involved in hematopoiesis, we are investigating whether there is reduced myelopoiesis and thus whether myeloid cells play a role as RVFV host cell and contribute to viral dissemination.

101. Ex vivo midgut cultures of Aedes aegypti: a new tool to study infections with mosquito-borne viruses and antiviral drugs

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Aedes aegypti mosquitoes are known vectors of chikungunya virus (CHIKV), dengue virus (DENV), and Zika virus (ZIKV). When blood-feeding on a virus-infected human, the mosquito ingests the virus into the midgut (stomach), where it must replicate and overcome the midgut barrier to disseminate to other organs and ultimately be transmitted via the saliva. Tools to study mosquito-borne viruses (MBVs) include two-dimensional cell culture systems and in vivo mosquito infection models, which offer great advantages, yet have some limitations. We describe for the first time the short-term ex vivo culture of Ae. aegypti midguts. Cultured midguts were viable for 9 days in a 96-well plate at 28°C and displayed peristaltic movements. Several MBVs efficiently replicated in the midguts: ZIKV, DENV, Semliki Forest virus (SFV) and CHIKV, as measured by qRT-PCR and end-point titration (for CHIKV-infected midguts). Fluorescence microscopy additionally revealed replication and spread of a fluorescent reporter virus (DENV2-mCherry) in the midgut. Finally, we evaluated the antiviral activity of the nucleoside analog, 7-deaza-2'-C-methyladenosine (7DMA), in the ex vivo midgut model. 7DMA reduced ZIKV viral loads with ~2 $\log_{10}$ at day 5 pi. A comprehensive screening of other antiviral drugs is currently ongoing. Together, our results show that ex vivo midguts can be efficiently infected with mosquito-borne flaviviruses and alphaviruses. Furthermore, the cultured midguts could potentially be used to evaluate the antiviral activity of inhibitors. Ex vivo midgut cultures could thus be a new model to study MBVs, offering the advantage of reduced biosafety measures compared to infecting living mosquitoes.

102V. Designing and Evaluating Neutralizing and Fusion Inhibitory Antiviral Peptides to a Tick-Transmitted Hemorrhagic Fever Virus

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Crimean-Congo Hemorrhagic Fever (CCHF) virus is a widespread, medically important, arboviral disease. Transmitted in enzootic cycles among ticks and vertebrates, it is endemic to Asia, the Middle East, Southeastern Europe, and Africa. Human infections are often limited to mild febrile illness, but severe disease may develop resulting in multi-organ failure, hemorrhagic fever, and high case fatality rates. It is a NIH/NIAID 'Biodefense - Category A' and WHO 'R&D Blueprint' priority pathogen. Despite this high consequence, no treatments are approved for CCHF. Antiviral inhibitory peptides, which antagonize viral entry, are licensed for human HIV infections and similar peptides have demonstrated in vitro and in vivo efficacies against high consequence viral pathogens. We designed inhibitory peptides against CCHF viral glycoproteins which contain areas critical to viral entry. Twenty-eight peptides were created with and without conjugation to sterols, which can enhance cellular uptake and antiviral antagonisms. These peptides were screened against a pseudotyped CCHF virus, to determine efficacies at low-containment (BSL-2) conditions. Plaque reduction neutralization tests, fusion assays, and cytotoxicity profiling (CT50) demonstrated that four peptide/sterol-groups inhibited pseudotype entry in vitro, with low CT50. These four peptides demonstrated neutralization on wild-type CCHF virus in maximum containment (BSL-4) and two of these peptides were further shown to inhibit the viral fusion process. This work represents the development of antiviral countermeasures targeting CCHF viral entry and provides a pseudotyped antiviral screening approach at low containment settings for this deadly human pathogen.

103V. Characterizing Humoral Immunity within the Henipahvirus Syrian Hamster Model

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Henipaviruses, such as Nipah and Hendra viruses, are zoonotic high-consequence pathogens due to their epidemic potential and associated high-case fatality rates. Few medical countermeasures are available to control Henipavirus infection in humans and additional development of countermeasures are warranted. To facilitate such developments, animal models have been established to evaluate experimental vaccines, antiviral treatments, and protective immune responses are warranted. The Syrian golden hamster (*Mesocricetus auratus*) is a reliable model of Henipavirus infection. While the hamster model capitulates much of human clinical Henipahvirus disease, there is a lack of immune

characterization for said model, which hinders mechanistic understandings of protection and antiviral countermeasure developments. To address this need, we sought to develop several immunodiagnostic assays using multiple Henipahvirus antigens. We evaluated naïve, succumbed, and survived hamster sera from Henipahvirus studies within our immunodiagnostic assays and were able to recognize seroconversion and were able to resolve humoral responses to Henipahvirus antigens. These studies offer insight into the humoral and protective responses to Henipavirus infections, within the Syrian golden hamster model, which may serve as an effective tool for developing antiviral countermeasures against these deadly diseases.

104. Remdesivir is efficacious in a hamster model of yellow fever infection and disease

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Yellow fever virus (YFV) is an mosquito-borne arbovirus that is endemic to tropical areas of Africa and South America. Despite the availability of an effective vaccine, YFV continues to cause periodic outbreaks of disease. No antivirals are currently approved for the treatment of yellow fever (YF) disease. Remdesivir (RDV) is a broadly active nucleotide analog prodrug that has been shown to be effective against various filoviruses, coronaviruses paramyxoviruses, pneumoviruses and flaviviruses in cell culture and in animal models. To broaden the range of efficacy in vivo, we evaluated the activity of RDV against YFV in a hamster model of infection and disease. Treatment with RDV at doses of 30 or 10 mg/kg/d administered beginning just prior to virus challenge and continuing for 7 days was effective in protecting hamsters from morbidity and mortality after YFV challenge. Survival, weight change, viremia and serum ALT were all significantly improved after prophylactic RDV treatment. Therapeutic administration of RDV was also effective in significantly improving survival when the 7-day treatment regimen with 30 mg/kg/d was initiated as late as 4 days after virus challenge, which corresponds with peak viremia in this model. These studies demonstrate prophylactic and therapeutic efficacy of RDV against YFV and suggest further studies towards clinical use are warranted.

[This work was supported in part by the NIAID, NIH contract HHSN272201700041I, Task A11]

105V. Heparin protects foetal human neural progenitor cells from ZIKA virus-induced cell death, while preserving their differentiation into mature neural-glial cells in vitro

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Background: The severe consequences of foetal ZIKA virus (ZIKV) infection have highlighted the need of antiviral agents for the treatment of infected pregnant women. We have previously shown that heparin, an anticoagulant used during pregnancy, inhibits ZIKV cytopathic effects (CPE) in hiPSC-derived neural progenitor cells (NPCs) without affecting viral replication (Ghezzi et al. 2017). Aim of this study was to explore heparin effects on ZIKV-induced CPE in human foetal neural progenitor cells (fhNPCs) in monolayer or in 3D system, i. e. neurospheres (fNS), and to determine whether heparin preserves the differentiation of ZIKV-infected fhNPCs into neural-glia cells.

Methods: Both fhNPCs monolayer and fNS were incubated with heparin 1 h prior infection with different ZIKV strains. CPE was determined by measuring the levels of adenylate kinase (AK) activity released in the culture supernatant and the diameter of fNS. After 1 week of differentiation, immunostaining of neural-glial cells was performed with specific antibodies for ZIKV, neurons (Tuji1), and astrocytes (GFAP).
Results: Heparin prevented ZIKV-induced CPE both in adherent fhNPCs or fNS as measured by the AK activity in the supernatant and diameter. ZIKV caused NS disruption that was preserved by heparin treatment. Importantly, even though ZIKV is detectable in heparin-treated fhNPCs, after 1 week of differentiation, heparin preserved the capacity of infected fhNPCs to mature into neural-glia cells.
Conclusion: Heparin could be a lead compound to discover derivatives for preventing ZIKV-induced cell death. The neuroprotection effect of heparin bears the potential to be an option to protect foetus development from ZIKV damage.

106V. Discovery of a chikungunya virus entry inhibitor targeting virus envelope protein

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Chikungunya (CHIKV) is an alphavirus that is transmitted to humans by Aedes spp. mosquitoes. In the last decade, CHIKV has expanded into tropical and subtropical areas around the globe and has posed an unmet need for vaccines and therapeutics. The E2-E1 envelope glycoprotein complex is located in the outer layer of the viral particle and mediates viral adsorption and entry into the host cell. In addition, E2-E1 has a known crystal structure thus constituting an attractive target for rational drug design. With the aim of identifying an inhibitor of E2-E1 function, we carried out a structure-based virtual screening targeting the envelope glycoprotein complex. The virtual screening was conducted on a druggable site located behind the fusion loop on E1 and was based on the consensus results of three different docking software. Through the virtual screening, we identified a specific inhibitor of CHIKV (compound 1, EC50 9.3 μM, CC50 >100 μM) and after a lead optimization process we obtained a compound with increased antiviral activity (compound 2, EC50 1.6 μM, CC50 56.0 μM). As expected given the proposed target, compound 2 inhibits the internalization step during CHIKV entry. In addition, we mapped mutations on the envelope proteins associated with partial resistance to the antiviral activity of compound 2. Furthermore, this compound specifically inhibited lentivirus pseudotyped with CHIKV envelope glycoproteins. These results suggest that compound 2 target is indeed the E2-E1 complex, encouraging further study on this series of molecules.

107. Gene expression profiling provides insights into the anti-dengue mechanism of metformin

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Dengue virus (DENV) is the cause of the most common mosquito-borne viral disease in the world, affecting an estimated 100 million people each year with potentially life-threatening acute illness. While specific antiviral drugs are currently being developed, drug repurposing has been attempted in the past but without success. Case-control study of diabetic dengue patients have suggested protective effect of metformin, a first-line anti-diabetic agent, against severe dengue. However, the mechanism of antiviral action has remained uncertain as this drug has pleiotropic effects in human cells. Using primary human monocyte-derived dendritic cells (moDCs), we found that pre-treatment with metformin inhibited DENV but not the closely related Zika virus infection. Full genome profiling of moDCs showed that metformin pre-treatment downregulated the oxidative phosphorylation (OXPHOS) pathway; Seahorse mitochondria stress test assay confirmed the switch of energy source from OXPHOS to glycolysis. Although the change in metabolism could impact DENV infection outcome, we did not find any difference in antiviral activity in 2 DENV strains that differed in how they alter glucose metabolism of infected cells. Instead, we found that, at 24-hours post-DENV infection, the top-most annotated expressed gene was a basic helix-loop-helix (BHLH)-related transcription factor. This transcription factor is known to be an important regulator of cell activity, including circadian rhythm. Our findings suggest a hitherto undefined antiviral pathway of metformin that is specific to DENV.

108. Molecular Mechanisms of Inhibition of Tick-borne Encephalitis Virus by Monoclonal Antibodies

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Tick-borne encephalitis (TBE) is a potentially lethal neuroinfection in humans, caused by TBE virus (TBEV), a member of genus *Flavivirus*, family *Flaviviridae*. Antibodies play an important role in control of TBEV infection, but the mechanisms of antibody-mediated TBEV neutralization remains largely unknown. We determined cryo-EM structures of the native

TBEV virion (strain Hypr) and its complex with Fab fragments of a neutralizing antibody at near-atomic resolution. Unlike most of the previously studied flavivirus-neutralizing antibodies, the Fab fragments did not lock the E-proteins in the native-like arrangement, but prevented the virus proteins from inducing membrane fusion in the endosome and releasing the viral nucleocapsid into the cytoplasm. Analysis of human antibody response to TBEV infection or vaccination revealed that expanded clones of memory B cells expressed closely related anti-envelope domain III (EDIII) antibodies in both cohorts, but the most potent neutralizing antibodies were found only in individuals who recovered from natural infection. These antibodies also neutralized other tick-borne flaviviruses. Structural analysis revealed a conserved epitope near the lateral ridge of EDIII adjoining the EDI-EDIII hinge region. Prophylactic or early therapeutic antibody administration was effective at low doses in mice lethally infected with TBEV. Antibody-resistant TBEV mutants were generated and characterized. The mutants had amino acid substitutions in EDIII and EDII, showed a small plaque size in mammalian cell culture and reduced levels of neuroinvasiveness in rodent models compared to the wild-type TBEV. The results demonstrate the importance of critical sites within the EDIII as determinants of virus virulence.

109V. Optimized Design, Synthesis and Biological Evaluation of 2-(4-(phenylsulfonyl) piperazine-1-yl)pyrimidine Analogues as Potent Inhibitors of Chikungunya Virus

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The identification of a hit by high-throughput screening exhibiting remarkable antiviral activity against this alphavirus led to a thorough structure-activity relationship study and the development of the CHVB compound series. Over 150 promising analogues of the initial hit were rationally designed and synthesized, and their antiviral effect was measured in the biological CPE-reduction assays. Starting from an EC_{50} of 8.68 µM a 20x fold improvement was achieved, resulting in a lower EC_{50} value of 0.47 µM and a higher security index of 172. To assess the best compound for future *in vivo* assays, additional extensive key *in vitro* investigations were performed with the most active and safe analogues. The intrinsic clearance in humane liver microsomes showed their good half-life time and metabolic stability. Furthermore, the compounds were assessed by their crucial ability to reach the patient`s circulation by an aqueous solubility assay. A cytotoxicity study in CaCo-2 cells showed remarkable low cytotoxicity. Additional extensive *in silico* investigations indicated logP values in an acceptable range for oral application as well as no foreseeable interactions with the hERG channel. In addition, an optimization of the established synthesis route was achieved. By changing the starting point of the synthesis route and by incorporating new protocols, the reaction time was shortened from 96.5 h to 18.5 h. Moreover, the products of two newly introduced protocols can be taken further without any purification needed – which also shortens the synthesis time by avoiding the time-consuming and challenging purification steps and increasing the overall yield.

110. Pyrimidine analogs as potential antiviral compounds against dengue and Zika viruses

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Dengue virus (DENV) and Zika virus (ZIKV) are vector-borne RNA viruses, known to cause human infections with similar symptoms and co-circulate in intertropical regions. DENV and ZIKV are assembled using the proteins C, prM and the envelope glycoprotein E. E protein is involved in the interaction of the virus with cell receptors and in the fusion of membranes. The crystal structure of DENV protein E (pdb ID: 1OKE) includes a hydrophobic pocket occupied by a detergent molecule n-octyl-β-D-glucoside (β-OG). The alignment of the sequences of the E proteins of DENV and ZIKV shows a high conservation of residues in the region of the β-OG pocket, which motivates the development of antiviral entry inhibitors with double action against the DENV and ZIKV. Previous studies identified molecules with a pyrimidine nucleus that presented EC50 values <1 µM against DENV. In this work, structural modifications were made in the pyrimidine nucleus and protein-ligand interactions were evaluated by docking simulations and molecular dynamics. Analogs substituted in position 6 of the leaders were synthesized, as well as compounds with triazine and thieno [3,2-d] pyrimidine. Subsequently, the in vitro antiviral activities of these compounds were evaluated by viral inhibition assays. Additionally, indirect immunofluorescence and RT-PCR were performed to corroborate the results obtained. The resulting EC50 values range from 10 µM to 0.7 µM for the different analogs. In conclusion, molecules with promising antiviral capacity against DENV and ZIKV were identified. The synthesis and evaluation of biological activity will continue with the optimization of these molecules.

111. Resveratrol and vitamins: modulators of Zika virus infection

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Zika virus (ZIKV) is an arbovirus belonging to the *Flaviviridae* family. Recently, we demonstrated that the Aryl Hydrocarbon Receptor (AHR), a cytoplasmic protein, is a pro-viral factor. Furthermore, AHR antagonists have been shown to be effective against ZIKV infection. Moreover, we have determined that resveratrol (RES), a phytochemical, inhibits ZIKV replication. The objective of the present work was to explore the possible interactions of AHR with 12 phytochemicals, including RES by molecular docking. It was found that vitamin E, vitamin A and xanthohumol exhibited binding energies of -13.23, -9.84 and -9.19 kcal/mol, respectively. Based on these results, we selected vitamins A and E to test their potential antiviral effect *in vitro*. After 48h of treatment with 800 µM of vitamin A, in Huh-7 cells infected with ZIKV, a 90% inhibition of viral yield was quantified. On the other hand, vitamin E did not show any effect on viral replication. According to this, and our previous findings, we also evaluated the antiviral activity of combined treatments of vitamin A (800 µM) with different concentrations of RES (12-100 µM). Huh-7 cells were infected and treated with several combinations during 48 h, and viral quantification was performed by PFU and RT-PCR assays. To note, the inhibition was significantly higher when both phytochemicals were tested together. In conclusion, this study demonstrated the positive synergy of RES and vitamin A on the inhibition of ZIKV, providing a new perspective for their use in infections caused by ZIKV.

112. The phenanthroindolizidine (-)-13aR-6-O-desmethyl-antofine inhibits Zika virus replication in human cells

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The medical importance of Zika virus (ZIKV) was fully highlighted during the recent epidemics in South Pacific islands and Americas due to its link with severe damage to foetal brain development and neurological complications in adult patients. Medicinal plants may be sources of antiviral phytocompounds. In this study, we performed a comparative study of the anti-ZIKV activity of 3 species of *Bohemeria* endemic or indigenous to Reunion Island. *Boehmeria stipulais*, *Boehmeria penduliflora* and *Boehmeria macrophylla*. Our results show that *B. macrophylla* and *stipularis* have a strong antiviral activity with IC_{50} of 0.1 µg/mL and 7.1 µg/mL respectively. However, *B. penduliflora* did not show an antiviral effect. Interestingly, time-of-drug addition assay show that *B. macrophylla* acts at the late stage of ZIKV replication while *B. stipularis* acts at the level of viral entry. Molecular networking analysis using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS²) highlighted the presence of a cluster of phenanthroindolizidine alkaloids family exclusively in *B. macrophylla* and a cluster of polyflavonoides in *B. stipularis*. Thus, A series of naturally occurring phenanthroindolizidine was tested against Zika virus in A549 cells. Our results show that (-)-13aR-6-O-desmethyl-antofine exerts a very potent antiviral effect with an IC_{50} of 0.003 pg/mL and a selectivity index of 8 million. Virological assays show that (-)-13aR-6-O-desmethyl-antofine acts at the late stage of the ZIKV life cycle inhibiting viral replication up to 8 hours post infection. Our results highlight the importance of medicinal plants as a promising source of natural antiviral compounds to prevent ZIKV infection.

113. Porphyrins as potent inhibitors of the entry process of tick-borne encephalitis virus

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Tick-borne encephalitis virus (TBEV) is an emerging human pathogen that causes potentially fatal disease with no specific treatment, although TBEV vaccines are available. Upwards of 10,000 TBEV cases per year worldwide are reported, with an increasing trend in recent years. Porphyrins are a large class of natural and synthetic organic heterocyclic molecules with tetrapyrrole structures. It is known that some of these molecules are potent inhibitors of viral infection due to multiple mechanisms of antiviral action. To elucidate these mechanisms against TBEV, we performed a series of virological assays. Based on our results, the main mechanism is blocking the adsorption of the virus to the

membrane of the host cell through incorporating in between viral membrane lipid molecules. Although, a decrease in temperature and the associated increase of membrane rigidity can eliminate the inhibition activity of some porphyrins. Another mechanism is probably incorporation into virions during maturation and disruption of their membrane, which resulted in the production of disrupted non-infectious particles. On the other hand, we have found that porphyrins can also inhibit viral infection after virus entry to the host cell. This could be possible by interacting with viral RNA because some porphyrins are known as guanine quadruplex binders. Although further analyses will be required, porphyrins represent promising molecules with multiple mechanisms of action. Exploration of these mechanisms is essential to deeper understand their antiviral actions to lead to the future development of novel, well-characterized antivirals.
Research project No. 20-20229S of the Czech Science Foundation is greatly acknowledged.

114. A Yellow Fever Virus NS4B Inhibitor Enhances the Activation of Multiple RNA Sensing Pathways and Induces Pre-mature Death of Infected Cells

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We previously reported a benzodiazepine compound BDAA that specifically inhibits yellow fever virus (YFV) replication in hamsters, with resistant mutation mapped to NS4B. In support of its preclinical development, we performed further mechanistic studies and found that BDAA enhances YFV-induced inflammatory cytokine response in association with the induction of dramatic ultrastructure alteration of viral replication organelles (ROs) and exposure of dsRNA in virus-infected cells using transmission electron and fluorescent microscope, respectively. These results support a model that BDAA interaction with NS4B may impair the integrity of YFV RO, which not only directly abrogates viral replication, but also promotes viral RNA releasing from RO to activate cytosolic RNA sensing pathways. Indeed, we demonstrated that BDAA treatment activates three dsRNA recognizing pathways, RLR, PKR and OAS-RNase L, in YFV-infected cells. While activation of RLR pathway depends on both RIG-I and MDA5, activation of OAS-RNase L pathway is mediated by OAS-3, but not OAS-1 or 2. Furthermore, we observed that BDAA treatment significantly accelerated YFV-induced cell death via caspase 8/9/3 mediated-apoptosis in a variety of cell types including primary human fibroblast cells. Studies are underway to map pathway(s) mediating BDAA-induced premature cell death using cell lines with single or multiple knockouts of RLR, PKR or OAS-RNase L pathway components. Taken together, BDAA primarily hits the YFV RO and executes multi-mode antiviral action including direct disturbing of viral replication, enhancing antiviral cytokine response, and premature killing of infected cells, which may collectively contribute to BDAA and its analog's potent antiviral effect *in vivo*.

115. Compound-A Improves Dengue Virus-induced Liver Injury in Immunocompetent Mice via Host Responses

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Dengue virus (DENV) infection is one of the most prevalent mosquito-borne viral diseases. Liver injury is observed in the severe forms of DENV infection; virus replication in the liver and hepatocyte apoptosis were the major contributing factors. The current study was aimed to investigate the efficacy of Compound-A (CpdA) or 2-(4-acetoxyphenyl)-2-chloro-N-methyl-ethylammonium chloride in DENV infection. CpdA treatment was found to restrict DENV production in DENV-infected HepG2 cells, but not in the liver of immunocompetent Balb/C mice model of DENV infection. CpdA treatment was found to benefit recovering leucopenia, thrombocytopenia, and liver transaminases in DENV-infected mice. Liver histopathology results were correlated with the liver transaminases suggesting improvements in the liver injury. Further, the expressions of 84 apoptosis-related genes in the liver tissue samples were characterized using a real-time polymerase chain reaction array profiler. A significant reduction in the expressions of pro-inflammatory cytokines was observed in the liver of DENV-infected mice when treated with CpdA. The treatment with CpdA in the DENV-infected mice was found to reduce the phosphorylation of MAPKs including p38-MAPK and JNK1/2. In addition, the

phosphorylation of their downstream kinases including MAPKAPK2, ATF-2, and HSP-27 was also found to be reduced by CpdA treatment. In conclusion, CpdA treatment reduces DENV replication in DENV-infected HepG2 cells, but not in the liver of DENV-infected mice; however, CpdA treatment modulates the host responses to improve DENV-induced liver injury in mice.

116V. Molecular basis of specific viral RNA recognition and 5′ end-capping by the Chikungunya virus nsP1

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Many viruses encode RNA modifying enzymes to edit the 5′ end of the viral RNA to mimic the cellular mRNA for effective protein translation, genome replication, and evasion of the host defence mechanisms. Alphavirus non-structural protein 1 (nsP1) displays both the N7-guanine-methyltransferase (MTase) and guanylyltransferase (GTase) activities to synthesise the 5′ end cap-0 structure of the viral genomic and subgenomic RNAs. Chikungunya virus (CHIKV) nsP1 assembles into a unique dodecameric crown-shaped structure, which hosts 12 copies of the putative active site for RNA capping. However, the molecular basis of the capping process remains unknown. We determined high-resolution cryo-EM structures of CHIKV nsP1 in complex with m7GTP/SAH, covalently attached m7GMP, and cap-0 viral RNA. These structures not only reveal the atomic-level details of viral RNA capping reactions but also uncover a sequence-specific virus RNA recognition feature. nsP1 recognises the primary viral RNA sequence and regulates viral RNA capping efficiency to ensure optimal genome replication and subgenomic RNA transcription. Furthermore, we discover that nsP1 has a novel mRNA decapping activity that could regulate the host gene expression.

117V. Targeting CHIKV Replication at the Viral Capping Enzyme nsP1 Through a Combination of Direct Inhibitors and Drugs Interfering With Lipid Metabolism

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Chikungunya virus (CHIKV), an arbovirus belonging to the Alphavirus genus, represents a global health challenge for which no antiviral drug has been approved. The mRNA capping process in CHIKV involving the viral nsP1 has been proposed as an appealing target based on significant differences with the homologous process in the host. Recently, it has been reported that nsP1 requires its anchoring to membranes organized as dodecamers for efficient mRNA capping. In infected cells, nsP1 has affinity for cholesterol-rich microdomains formed at the plasma membrane, depending on the palmitoylation of its central domain and drugs interfering with lipid metabolism (i.e cerulenin, orlistat or imipramine) significantly impair CHIKV replication. Here we have tested whether the combination of MADTP compounds, described as the first CHIKV nsP1 inhibitors, with these drugs may have an additive or synergistic effect in inhibiting CHIKV replication. Our results demonstrate that the combination of MADTP410 with orlistat using HEK293T cells infected with CHIKV (LR-OPY-1 strain) is able to abolish CHIKV replication at low concentrations of both drugs. A similar effect was observed with imipramine and confirmed with HFF-1 cells. These results constitute a good example supporting that the combination of direct- and host-targeting antivirals may result in an efficient way to inhibit CHIKV replication while reducing the individual doses of the drugs used. Funded by grant PID2019-105117RR-C22 from Agencia Estatal de Investigación MCIN/ AEI / 10.13039/501100011033 and ANR-18-CE11-0026-01 funding from French Agence Nationale de la Recherche.

118V. A Pre-Membrane Protein PrM D29V Substitution Attenuates a Clinically Tested Live Dengue Vaccine

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Dengue virus (DENV) is an *Aedes* mosquito-borne flavivirus that has emerged to be the most important mosquito-borne viral disease globally. A tetravalent dengue vaccine development has been challenging due to insufficient knowledge on DENV pathogenesis. Understanding the genetic and molecular basis of attenuation will allow us to develop a targeted mutagenesis approach to derive all 4 attenuated DENVs that is sufficiently safe but immunogenic. In addition, this knowledge will also be useful in the identification of host dependency factors that can be targeted for the development

of antiviral therapeutics. We have been studying the molecular properties of a live attenuated DENV2 strain, PDK53, which was derived through 53 serial passages of wild-type 16681 strain, in primary dog kidney cells. PDK53 has successfully completed a phase-3 clinical trial, and differs from its parental 16681 strain by only five amino acid substitutions and one consensus mutation in the 5′ untranslated region of the genome. Using site-directed mutagenesis on a 16681 infectious clone, we identified the aspartate-to-valine substitution in the pre-membrane protein that that attenuated 16681 in mammalian cells but not mosquitoes. Using genomics coupled with proteomics approaches, we found that prM D29V resulted in the loss of binding of prM to HMGB1, a cytokine mediator of inflammation. Our study implicates the interaction of HMGB1 with the prM as an important determinant of virus pathogenesis.

119V. Development of zika virus stable replicon cells expressing secretory luciferase

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Zika virus (ZIKV) is a flavivirus (Flaviviridae family) and is transmitted to humans by infected Aedes mosquitoes. The ZIKV epidemic in the Americas in 2015 established ZIKV as a major international public health threat and uncovered its association with severe diseases, including microcephaly and Guillain-Barré syndrome. However, specific antiviral drugs or licensed vaccines against the pathogen are not available. In this study, we developed a ZIKV replicon cells expressing secretory luciferase for high-throughput screening of anti-ZIKV compounds. An infectious molecular clone of ZIKV, African lineage MR766 strain based rZIKV-MR766/pMW119-CMVP, was used to construct the replicon. Major segments of genes encoding the structural proteins were replaced with a fragment containing a fusion of the gene encoding Gaussia luciferase, FMDV2A cleavage site, neomycin-resistance gene, and EMCV internal ribosome entry site. After transfection of the replicon RNA into Huh7 cells (human hepatoma cell line), transfected cells were selected by geneticin treatment. Selected clones exhibited high levels of luciferase activity in the culture supernatant and expressed NS proteins in the cells. It could be maintained at least two months after transfection of replicon RNA in the presence of geneticin. Furthermore, the luciferase activity was significantly reduced by anti-ZIKV inhibitors and siRNA targeting ZIKV genome RNA. The subgenomic replicon cells expressing a secretory luciferase gene will be useful for high-throughput screening of anti-ZIKV compounds and analysis of the replication mechanisms of the ZIKV genome.

120V. Transcriptional response to dengue virus infection reveals potential host targets for antiviral intervention

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Dengue virus (DENV) is a global health concern for which no antiviral treatment exists. DENV infects cells from the mononuclear lineage (i.e., dendritic cells [DCs], macrophages, and monocytes [Mo]), which play an essential role in the antiviral response but also contribute to DENV pathogenesis. We performed a whole transcriptome and cytokine profiling of human primary mononuclear cells and compared cells 16 hours post-DENV-2 infection, with and without antiviral treatment (2′-C-methylcytidine [2′CMC]) to uninfected controls to identify host markers associated with infection as potential antiviral targets.

The transcriptomic profiles of infected monocyte-derived (md)DCs, macrophages type 2 (MΦ2), and Mo demonstrated an alteration in pathways associated with "response to type I interferon" and "negative regulation of viral processes". Specific pattern recognition receptor and chemokine genes were significantly upregulated in these three cell types. The protein overexpression of CXCL11, IP-10, MCP-2, and MIG was confirmed using a Luminex assay. Compound 2′CMC inhibited DENV replication and revealed genes highly dependent on the percentage of infected cells: TRAIL (TNFSF10), MIG (CXCL9), I-TAC (CXCL11), OASL, ISG20, RSDA2, IDO1. Evaluation of compounds targeting these proteins led to the identification of JNJ-16-IDO, which targets IDO1 and potently inhibits DENV-2 replication in all three cell types (EC_{50} of 2.4 μM and absence of cytotoxicity). In summary, we present a comparative transcriptional response to DENV-2 infection in human primary mdDCs, MΦ2 and Mo through which we identified IDO1 as host dependency factor for DENV replication. JNJ-16-IDO, targeting IDO1, displayed antiviral activity and represents a potential antiviral candidate.

121V. Potent Inhibition of Zika Virus Replication by Favipiravir in HeLa Cells is Linked to Substantial Alterations in Viral Infectivity

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Zika virus (ZIKV) is a mosquito-borne flavivirus associated with an increased risk for congenital abnormalities in neonates and neurologic complications in children and adults. Although ZIKV infection can substantially impact patient development and quality of life, there are currently no approved antiviral therapies or vaccines to treat or prevent infection. This study aims to evaluate the antiviral potential of the nucleoside analogue favipiravir (FAV) against ZIKV in two clinically relevant human cell lines. HeLa (cervical) and SK-N-MC (neuronal) cells were infected with ZIKV and treated with increasing concentrations of FAV. Viral supernatant was sampled daily and infectious viral burden was quantified by plaque assay on Vero cells. Changes in ZIKV infectivity were measured by calculating viral specific infectivity. FAV potently inhibited ZIKV in HeLa cells and the calculated EC_{50} value in this cell line (247.3 uM) is therapeutically achievable. Robust activity in HeLa cells is likely linked to drug induced changes in viral infectivity since treatment caused infectious titers and viral infectivity to decrease in a time and concentration dependent manner. In contrast, viral suppression achieved in SK-N-MC cells was modest (EC_{50} 388.8 uM) and FAV induced alterations in specific infectivity were slight in this cell line. These results demonstrate FAV ability to substantially alter viral infectivity is cell line dependent and suggest FAV's robust anti-ZIKV activity in HeLa cells is related to the strong effect on viral infectivity observed in this cell line. We are actively working on characterizing host cell factors that explain differences in this infectivity phenomenon.

122V. Identification of Druggable Host Factors Crucial for Flavivirus Replication

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RNA viruses are the etiological agents of many recent outbreaks and epidemics of viral origin, and include endemic viruses such as dengue virus (DENV) and re-emerging ones such as Zika virus (ZIKV). There are no effective antiviral therapeutics or vaccines against many of these viruses. While antiviral drug development has traditionally been directed at virus targets, host factors and pathways have now emerged as effective targets for antivirals. Current methods to identify antivirals rely heavily on blind large-scale screens. We have taken a more directed approach by identifying host factors that interact with virus proteins and/or pathways that are perturbed during virus infection. Through a transcriptomic approach, we demonstrate that ZIKV infection induces an inflammatory gene signature that we have now found to be driven by the NF-kB family of transcription factors. In addition, we have identified both known and novel host protein interactors of DENV proteins in the context of virus infection via a mass spectrometry-based proteomic approach. These interacting host factors are involved in a variety of host pathways. By targeting these host factors and pathways using RNA interference and small molecules, we have revealed their functional significance to the replication of these viruses.

135. Universal Virucidal Drugs

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SARS-CoV-2 spread in China in December 2019 and has caused a global pandemic, with almost five million deaths. Simultaneously, we are still fighting other viruses, such as influenza, which due to the absence of a universal vaccine, can lead to medical and economic impacts. There is a need to develop new platforms for the creation of antiviral drugs that could potentially inhibit the infectivity of many different viruses via minimal modifications. Recently, our group developed macromolecules capable of targeting the receptor binding domain (RBD) of many viruses. Importantly, these compounds showed irreversible infectivity inhibition due to permanent disruption of the viral capsid. These compounds have shown very limited toxicity and in vivo efficacy against a good number of viruses, yet their ability of targeting a virus is limited to simple mimicking of glycans on the cell membrane. Such rather unspecific targeting could lead to dangerous side-effects in vivo. In this work we will show that similar molecules can be developed in a way that

maintains the same mechanism of action but that targets specifically different parts of the virus. Various viruses have been considered and preliminary results show that molecules targeting the stem region of influenza-virus hemagglutinin show no cytotoxicity and are able to irreversibly inhibit influenza replication in vitro, with an IC_{50} of 0.07 ug/mL, and ex vivo, on human respiratory tissues. If the approach will be confirmed with other viruses, we will have generated a universal design for virucidal drugs.

137. Treatment of a chronic human norovirus infection of an immunocompromised patient with favipiravir and nitazoxanide: assessing drug efficacy and the mechanism of action using a zebrafish larval model

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As ~18% of transplant patients develop chronic human norovirus (HuNoV) infections, there is an urgent need for treatment. Yet today there is no approved antiviral therapy or prophylaxis, and thus experimental treatment remains the only option. A pediatric patient with severe combined immunodeficiency developed a chronic HuNoV (GII.P16-GII.4) infection with persistent diarrhea, significant weight loss, and parenteral nutrition-dependency after hematopoietic stem-cell transplantation. Compassionate treatment with favipiravir and nitazoxanide was started, two broad-spectrum drugs with *in vitro* evidence of anti-norovirus activity (while *in vivo* efficacy data is lacking).

Stool samples of the patient were collected before and during antiviral treatment. After inoculating zebrafish with a pre-treatment stool sample, favipiravir and tizoxanide reduced HuNoV RNA levels by 1 and 1.5 log_{10}, respectively. Upon treatment with tizoxanide, overexpression of IRF-1 and Mx confirmed an immune-boosting antiviral mechanism. Zebrafish were inoculated with serial dilutions of pre- and post-treatment stool samples to determine the 50% infectious dose (ID_{50}). The ID_{50} increases from 166 to 401 and 620 viral RNA copies after, respectively, one and six months into treatment, while a typical mutagenesis signature of favipiravir was found upon sequencing (i.e., accumulation of mutations in the viral genome, particularly AG/CT mutations). Parenteral nutrition was successfully weaned and weight gain improved. We demonstrate that treatment is linked to an accumulation of mutations resulting in loss of infectivity, despite a small reduction in viral load. Moreover, a threshold of accumulated mutations in the viral genome could be used as a biomarker of successful antiviral treatment with mutagenic antivirals.

138. Antiviral Activity of 1-O-alkyl-2-O-aryl-sn-glyceryl-3-P-RVn Compounds Against RSV-A2 in Hep-2 and HeLa Cells

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Respiratory Syncytial Virus (RSV) infection accounts for ~58,000 hospitalizations per year of children < 5 years of age and for 14,000 yearly deaths of those >65 years of age. Monoclonal antibody prophylaxis is available for high-risk infants but no preventive vaccine or antiviral therapy is currently available.

The broad-spectrum antiviral GS-441524 (RVn) and its more potent prodrug, remdesivir (RDV), are effective RSV polymerase inhibitors, however, RDV requires inconvenient intravenous administration and has a short plasma half life. To improve its oral absorption and cellular uptake, we modified RVn-5'-monophosphate (RVn-P) with various 1-*O*-alkyl-2-*O*-aryl-*sn*-glyceryl lipids as shown in the Figure. Compound 1 demonstrated plasma concentrations of 1 higher than the EC_{90} 12 hours after oral administration to Syrian Hamsters and it is converted intracellularly to the active triphosphate (Schooley RT, et al. *Antimicrob. Agents Chemother.* 2021 65, e01155-21). The new analogs of RVn-P were tested against RSV-A2 in HeLa or Hep-2 cells by CPE reduction, and cytotoxicity was determined by Cell Titer Glo. EC_{50} values of compounds 1, 2 and 3 ranged from 5 to 10 nanomolar in Hep-2 cells with selectivity indices as high as 2500 (compound 3). Our data support the development of lipid prodrugs of RVn monophosphate as potent oral antivirals that could be used to prevent or treat RSV infections.

139. In Vitro and In Vivo Activity of Antimicrobial Peptoids Against Herpes Simplex Virus 1 and 2

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HSV-1 and HSV-2 cause infections in the oral cavity, affecting millions each year. Few antiviral drugs can effectively treat these infections. Antimicrobial peptides (AMPs) represent a novel source of potential broad-spectrum antiviral drugs, inactivating enveloped viruses through the disruption of their viral envelopes. Development of AMPs as antimicrobial therapeutics is hampered by many factors, especially the peptide structure, which is vulnerable to proteases *in vivo*. To circumvent this problem, sequence-specific *N*-substituted glycine oligomers called peptoids were developed. In addition to being resistant to proteases, peptoids also exhibit increased stability. We examined the antiviral potential of these peptoid mimics of AMPs, both *in vitro* and *in vivo*. Antiviral assays on an oral keratinocyte cell line were performed by incubating 0-20 µg/ml of each peptoid with 1 x 10^4 pfu HSV from 30 min to 2 hr, then adding the treated HSV to TIGK cells at an M.O.I of 0.1 (control) for 24 hr prior to HSV DNA analysis by qPCR. Peptoids exhibited potent *in vitro* antiviral activity against both HSV-1 and -2 when incubated prior to infection. Examination of the activity at different points during viral infection demonstrate that the peptoids act directly on the virion, in the pre-attachment phase. When applied to the scarified lip of BALB/c mice 2 days after infection, 50 µg/ml increased the healing rate, and prevented development of visible lesions and migration to the trigeminal nerve. The data suggest that peptoids represent a new class of effective broad-spectrum antiviral agents for oral herpesvirus infections.

140. Elucidation of the Mechanism of Action of Various Flex-Acyclovir Analogues

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As outbreaks of new viruses occur, the need for broad-spectrum antiviral drugs has increased. In that regard, nucleos(t)ide analogues have a rich history as antivirals. One modification in nucleoside drug design that has proven successful is the use of acyclic sugars, such as that found in Acyclovir (ACV), an FDA-approved drug for herpes simplex virus. Research in the Seley-Radtke group has focused on the development of novel nucleos(t)ide analogues known as "fleximers", which feature a "split" purine nucleobase, where a carbon-carbon single bond connects the pyrimidine and imidazole rings, thus introducing flexibility to the nucleobase scaffold. This endows the "fleximers" with potent activity not seen for the corresponding rigid analogues. Combining the flex-nucleobase with the acyclic sugar of ACV produced a series of doubly flexible Flex-ACV analogues. These novel analogues have exhibited low micromolar to nanomolar levels of activity against human coronaviruses (SARS-CoV-1, MERS, HuCoV-NL63) as well as Ebola, Yellow Fever, Dengue and TBEV, while ACV has no activity against those viruses. Recently Flex-ACV analogues have shown mid-micromolar activity against SARS-CoV-2. An important aspect of nucleoside drug design is mechanism of action (MOA) studies to evaluate how antiviral activity occurs. Following these observations, Flex-ACV analogues HP-083, HP-083-McG, HP-083-MP, and HP-083-TP are being utilized in MOA studies to elucidate enzymes that the Flex-ACV analogues interact with, such as that of CapA and HINT, which have been determined to remove the prodrug on HP083-McG. SARS-CoV-2 RNA dependent RNA polymerase (RdRp) assays have also been carried out. The results are reported herein.

141. Hits on the Virus RGB Palette

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Flaviviruses such as dengue (DENV), yellow fever (YFV) and Japanese Encephalitis viruses (JEV) pose a significant threat to the world. Potent antivirals, ideally with activity beyond specific viruses (i.e. pan-flavivirus inhibitors) are urgently needed. To screen for such broad-spectrum inhibitors, we need novel approaches in drug discovery. To this end, we developed a multiplex antiviral assay that is based on the visualization of mixed infections by high content imaging (HCI) using a selection of recombinant flaviviruses tagged with different fluorescent proteins (FPs). Based on brightness and spectral separation of a range of FPs, we combined YF17D/mCherry (Red, R), JEV/GFP (Green, G), and DENV2/Azurite (Blue, B) for mixed infection experiments on target cells constitutively expressing a far-red FP. For assay validation and proof of concept for imaged-based antiviral screening, two known pan-flavivirus inhibitors (IFNα and NITD008) and a highly DENV-specific inhibitor (3-Acyl-indole) were used. All assays were run in an automated combined robotics-biosafety containment system. Both approaches, individual single virus assays as well as the novel multiplex assay yielded similar inhibition and sensitivity profiles. To accelerate data analysis and to visualize individual inhibition spectra, we developed a kernel based on the RGB paradigm that allows to deconvolute our multidimensional quantitative data into a simple color code per condition tested. Such approach will provide color-coded coordinates in a 3D-plot visualizing broad spectrum activity and/or selectivity towards certain members of the flavivirus family tested. Our novel approach may provide efficient means to find potential pan-flavivirus inhibitors to curb current and future flavivirus outbreaks.

142. Broadly active antiviral polyphenolic compounds inhibit SAR-CoV-2 in culture

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We have developed a family of poly-polyphenols that inhibit the infectivity of HSV-1 and IAV virions, which attach to glycosamino- or sialylated-glycans (GAG, SG), respectively with up to submicromolar concentrations. As glycan attachment is conserved among all respiratory human viruses, we had proposed that these compounds could be used as first line defense against emerging respiratory viruses. When the SARS-CoV-2 pandemic started, we screened 25 of these compounds against an endemic human coronavirus, OC-43. With the exception of the catechin EGCG, all compounds that had inhibited the infectivity of HSV-1 or IAV scored as hits in a CPE screen for inhibitors of viral spread, but as expected negative in a screen for inhibitors of viral replication; none of the hits was cytotoxic. Fifteen compounds were selected to test their effects on OC43 burst assays at low multiplicity, including EGCG. Twelve had EC50 below 40 μM; none were cytotoxic and one showed cytostatic effects at 100 μM. Eight compounds were then tested against SARS-CoV-2 burst size at low multiplicity of infection. The three most potent ones had an EC50 in the submicromolar range, and two others in the low micromolar range. The common structural features among the compounds that have the best potency against the most viruses include a rigid planar linker core or more than two polyphenol rings. Potencies against SARS-CoV-2 are more closely related to potencies against HSV-1, and those against OC43, with those against IAV, suggesting that these two coronaviruses differ in their preferred glycan binding.

143. PRTX007, a TLR7 agonist, demonstrates broad-spectrum antiviral activity and is appropriate for pandemic preparedness

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We have experienced devastating viral outbreaks involving emerging viruses such as SARS coronaviruses, Zika, Dengue, and Ebola. Despite the existence of some successful antivirals, there are limited small molecule, broad-spectrum

drugs against emerging viruses. Toll-like receptor 7 (TLR7) is a key sensor of viral infection, and engagement leads to direct activation of plasmacytoid dendritic cells (pDCs) and B cells. A major limitation in targeting TLR7 is inflammation accompanying activation of two key intracellular pathways in pDCs, leading to biosynthesis of (1) all human Type I/III interferons (IFN) and (2) NF-kB–mediated proinflammatory factors. Here we report the broad-spectrum in vitro antiviral activity of PRTX007, a novel TLR7 agonist prodrug and PRX034, its corresponding systemically acting drug. PRTX007 is the product of an extensive medicinal chemistry program at Primmune Therapeutics. We have successfully decoupled the link between NF-kB activation and induction of IFN biosynthesis as shown in preclinical and in clinical studies of healthy volunteers. PRX034 conditioned media inhibits 8 RNA viruses in vitro (Table), including SARS-CoV-2, at a potency 107-fold greater than what would be expected by external IFN. Activity has been demonstrated in murine models of viral infection. PRTX007's MOA makes it uniquely suitable as a clinical candidate to successfully treat acute viral infections without exacerbating inflammatory pathology or being subject to development of antiviral resistance. This small molecule drug is orally administered and could be readily distributed to the population in need. Lastly, PRTX007 can be synthesized on a large scale and is suitable for stockpiling and pandemic preparedness.

144. Mining Chemical Bioactivity Data to Identify Broad-Spectrum Antiviral Agents

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Diseases caused by humans' yearly exposure to new viral treats costs millions of lives and trillions of dollars in damage to the global economy. Despite the rapid development of vaccines for SARS-CoV-2, the lack of small molecule antiviral drugs working against families of viruses (broad-spectrum antivirals) left us vulnerable between the beginning of the outbreak and the widespread availability of vaccines. Developing broad-spectrum antivirals is an attractive, yet challenging, approach that could prevent the next, inevitable, viral outbreak from becoming a global catastrophe. To explore the potential for the discovery of broad-spectrum antivirals using a data-centric approach, we (i) identified, collected, curated, and integrated all chemical bioactivity data available in ChEMBL for 30 emerging viruses documented as potential treats to global human health; (ii) identified and solved challenges in data reproducibility including assay ambiguity, missing information, and incorrect annotations; (iii) developed a publicly available, FAIR-compliant database of compounds tested in phenotypic (119,007 entries) and target-based (117,705 entries) assays for these viruses; and (iv) identified compounds showing broad-spectrum antiviral activity. We identified eight compounds active against 3-4 viruses from phenotypic data, 16 compounds active against 2 viruses from target-based data, and 35 compounds active in at least one phenotypic and one target-based assay. The final database that we called SMACC (Small Molecule Antiviral Compound Collection) contains over 236,000 entries for 30 viruses and may serve as a reference for virologists and medicinal chemists developing broad-spectrum antiviral agents in preparation for future viral outbreaks. The SMACC database is publicly available at **https://smacc.mml.unc.edu**.

145V. Ribavirin enhances the anti-coronavirus activity of Molnupiravir and GS-441524 in ex vivo model

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To aid in the search for broad-spectrum anti-coronavirus, we explored in the human airway epithelial cells (HAEC) the antiviral effect of a triple combination of ribavirin 10 µM, molnupiravir 1 µM, and GS-441524 1 µM (parental nucleoside of remdesivir). The cultures were pre-incubated with mono-, dual- or triple therapy at a suboptimal dose of each compound and then apically infected with clinical hCoV-OC43 strain. Treatments were refreshed every other day until day 6 p.i. The shedding of viral RNA in the apical washes, the trans-epithelial electrical resistance (TEER), and the release of lactate dehydrogenase (LDH) in the basal medium were monitored until day 10 p.i. While no inhibition of single treatment was observed within 10 days, a triple therapy resulted in a remarkable protection with 99.5%

decrease (2.3 log) in vRNA level until day 8 p.i., followed by a viral rebound on day 10 p.i.. In an independent experiment, antiviral activities between single, dual, and triple therapies were compared. Molnupiravir, GS-441524, and ribavirin monotherapies showed < 20% inhibition against hCoV-OC43 (< 0.65 log) whereas GS-441524/ribavirin, GS-441524/molnupiravir, ribavirin/molnupiravir, and GS-441524/ribavirin/molnupiravir led to 46%, 29%, 15%, and 66% viral inhibition, respectively. The synergistic interactions of GS-441524/ribavirin or GS-441524/ribavirin/molnupiravir were confirmed by the Bliss interaction model. To the best of our knowledge, this is the first study demonstrating that the combination of nucleoside analogs results in a synergistic protective activity. Our finding provides pre-clinical evidence for testing the efficacy of combination remdesivir/ribavirin/molnupiravir.

146. Development of a high-throughput screening pipeline for potential inhibitors of SARS-CoV-2 replication using the Caps-It system, an automated lab-in-a-box

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Major outbreaks of infectious diseases have occurred globally in the last 2 decades. The ongoing COVID-19 pandemic is the prime example of this and has caused major disturbances throughout the world. Despite of having developed efficient vaccines, there is still a need for antiviral therapies to combat this pandemic. In early 2020, we established a highly efficient in vitro antiviral compound screening campaign against SARS-CoV-2. A straightforward CPE based assay was developed using a VeroE6-eGFP cell line and a SARS-CoV-2 strain recovered from the first Belgian patient returning from Wuhan. Infected cells die leading to a strong decrease of fluorescent signal. We have used this assay to collect over 2 million data points using our own in-house compound libraries and repurposing as well as drug discovery libraries from several partners. Interesting hits serve as starting point for hit-to-lead campaigns. The screenings campaign was performed in our lab-in-a-box, the Caps-It. This system contains a fully integrated network of automated robotic laboratory instruments inside a 50 m^3 BSL-3+ isolator facility. By utilizing the automation capacity of the Caps-It we screened over 20.000 compounds each day while retaining a high qualitative standard ($Z' > 0.8$). The high-throughput screening (HTS) capabilities of the Caps-It system has proven to be a compelling and efficient tool during this pandemic. We believe that the Caps-It could be deployed for future HTS programs on pathogens with higher biosafety levels to prepare against future outbreaks.

147V. Design and Synthesis of Flex AT-527 as a Potential Antiviral Therapeutic

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Due to the COVID-19 pandemic, the need for broad-spectrum antivirals to treat infected individuals has been illuminated, even with vaccines available to the general population. Nucleoside analogues are sought after for this reason, as they have shown significant activity as a class of antivirals for decades. The Seley-Radtke group has focused on modifying the bicyclic purine base moiety of nucleoside analogues by incorporating a carbon-carbon single bond between the two heterocyclic components, endowing the nucleobase with flexibility. As a result, the fleximers can adopt a variety of favorable conformations thereby allowing the compound to exhibit potent antiviral activity not seen in the rigid-parent nucleoside. In addition, this allows for the ability to overcome antiviral resistance, and be recognized by atypical enzymes. This has led to significant activity against a wide variety of viruses. AT-527 is a nucleoside analogue originally designed to treat Hepatitis C Virus (HCV), that has shown activity against SARS-CoV-2 and other viruses. The aim of this project is to incorporate the fleximer technology to the AT-527 scaffold, thereby potentially expanding its biological scope. Computational docking studies were carried out to explore the binding potential for a series of AT analogues, as well as to guide future SAR studies. The synthesis of the parent Flex AT-527 was completed in ten steps, with each step having fair to good yields. The results of this project are reported herein.

148. Single Infectious Unit Sequencing Revealed That Alphaviruses Maintain Their Infectious Populations Within a Narrow Genetic Heterogeneity Range

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Knowledge of RNA virus population structure is important to develop lethal mutagenesis as an antiviral strategy. Estimating virus mutation frequencies using the total population may not reveal the true genomic diversity of infectious populations due to bias towards sequences from the fittest or, conversely, non-viable populations. To understand how an infectious virus population undergoes lethal mutagenesis, we developed a single-infectious unit sequencing approach and analyzed changes in the genetic structure of Venezuelan Equine Encephalitis Virus (strain TC-83) population following treatment with β-d-N4-hydroxycytidine (rNHC), an RNA mutagen with potent antiviral activity. We found that the mutation frequency of the normal infectious TC-83 population is 10-fold lower than that of the total population (0.2 and 2.82 per 10E4 n.t., respectively). The mutation frequency of the total population increased in a dose-dependent manner with rNHC treatment (6.8, and 43.3 per 10E4 n.t., at 1, and 50 μM, respectively). However, rNHC's antiviral effect peaked at 10-20 μM. On the other hand, the mutation frequency of infectious TC-83 population peaked at 6.3 per 10E4 nucleotides at > 10 μM of rNHC, which is significantly lower than that of the total population but still reflects rNHC's antiviral activity pattern. We also found that a certain percentage of the population remained mutation-free, which explains the survived population at higher rNHC concentrations. Our study showed that alphaviruses maintain their infectious populations within a narrow genetic heterogeneity range and that lethal mutagenesis may not be able to inactivate the entire population when it is big.

149. Synthesis and Biological Evaluation of a Flexible Nucleoside Analogue of AT-527

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Nucleoside analogues have long served as a source of antiviral therapeutics due to their ability to disrupt numerous biological functions that are required for viral replication. The SARS-CoV-2 pandemic has shown the need for proactive pandemic preparedness by having a large and continuously developing arsenal of antiviral therapeutics. The Seley-Radtke lab focuses on the development of such compounds, utilizing "Fleximer" technology to produce nucleosides with flexible nucleobases, featuring a split purine base moiety. This modification allows for potential alternative binding conformations, atypical enzyme recognition, antiviral activity in viruses that the Fleximer's rigid counterpart is inactive against. Fleximers also have the potential to overcome steric and electronic clashes within enzyme active sites that give rise to antiviral resistance via point mutations. The project reported here-in describes the synthesis and biological evaluation of a Fleximer nucleoside analogue of AT-527, an experimental nucleoside therapeutic that has shown promise in early clinical trials. The total synthesis currently occurs across 10 steps with very good to fair yields for each step. Maximum tolerated dose (MTD) studies performed in mice show that single doses up to 300 mg/kg did not show any adverse effects. A lack of cytotoxicity in cells has also been shown. Antiviral testing against several viruses, as well as structure activity relationship (SAR) studies are currently underway to further explore these analogues.

150. Design, Synthesis, and Enzymatic Activation of the Nucleobase Analogue T-1105

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The RNA-dependent RNA polymerase (RdRp) is one of the few common structural proteins shared among the vast array of RNA viruses and therefore makes a tempting target for therapy to curtail viral endemics (and possible pandemics). Nucleotides, nucleosides, and nucleic acid analogues have a proven history as effective antiviral compounds and comprise the backbone of several therapeutic strategies including those for HIV and HCV. The nucleic base analogues T-705/-1105 have demonstrated antiviral activity against a wide range of RNA viruses including Ebola, influenza, and coronaviruses (including SARS-CoV-2). Mechanism of action studies have demonstrated that the nucleoside triphosphate of these analogues is recognized as a substrate by the RdRp resulting in inhibition of RNA polymerase activity and viral replication. Moreover, nucleoside di- or nucleoside triphosphate prodrug of the Di*PPro*- or Tri*PPPro*-class showed improved antiviral activity when compared to the parent nucleobases. However, the mechanism by which

these prodrugs are enzymatically converted to an active triphosphate compound is incomplete. Previous studies have demonstrated that the endogenous mammalian enzyme HGPRT is responsible for the conversion of the nucleobase prodrug into the corresponding nucleoside monophosphate. However, *cyclo*Sal-nucleoside monophosphate prodrugs of these nucleobases failed to retain antiviral activity in HGPRT-deficient MDCK-cells indicating additional metabolic bottlenecks beyond traditional nucleoside analogue activation. In addition, the remaining enzymatic processes for the conversion of the monophosphate to active triphosphate remain unclear. To that end, we examined the conversion of T-1105-MP to an active triphosphate compound; the results to date are presented herein.

151. Broad-spectrum Inhibitors against the SARS CoV-2 NSP14 and flavivirus methyltransferases

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Coronaviruses (CoVs) are broadly distributed in humans, bats, and other mammals, and are adept at host-species movement, as demonstrated by the recent emergence of SARS-CoV in 2003, by the recent identification of MERS in 2012, and by most recent emergence of SARS CoV-2 in 2019. Flaviviruses, which are primarily insect-borne, are also associated with significant worldwide morbidity and mortality and have been found on every inhabited continent. Unfortunately, current therapeutic options for treating diseases associated with these viruses are limited. All flaviviruses and CoVs encode methyltransferases (MTases)—flaviviral NS5 for both N-7 and 2′-O methylations of viral genomic RNA, CoV/CoV-2 NSP14 N-7 MTase, and CoV/CoV-2 NSP16/NSP10 2′-O MTase. The N-7 MTase function is essential for replication of the viral RNA genome, whereas 2′-O MTase function is required for the virus to evade the host innate immune response. These activities are conserved among the flaviviruses as wells as CoVs. Here we developed a novel high throughput screening assay to identify innovative inhibitors against the SARS-CoV-2 NSP14 and flavivirus MTases. Several candidate inhibitors were identified to potently inhibit the binding of co-factor SAM to the MTases and inhibit the MTase activities. Among these candidate inhibitors, two showed significant inhibition against SARS-CoV-2 and Zika virus in cell-based antiviral assays. Overall these molecules are promising candidates to further develop as broad-spectrum antivirals against multiple viruses.

152V. Design, synthesis and biological evaluation of LAVR-289, a new broad-spectrum antiviral ANP

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Acyclic nucleoside phosphonates (ANPs), such as (R)-PMPA [9-[9(R)-2-(phosphonomethoxy)propyl]adenine] and PMEA [9-[2-(phosphonomethoxy)ethyl] adenine] discovered by A. Holý and E. De Clercq in 1986, led to a new family of nucleotide analogs which has attracted considerable attention. In order to improve the oral absorption of these phosphonate analogs, ANPs are delivered as prodrugs such as acyloxyalkylester (pivaloyloxymethyl, POM), or ((isopropyloxycarbonyl-oxymethyl)-ester, POC), alkoxyalkyl groups (hexadecyloxypropyl, HDP), and more recently on phosphonoamidates (ProTides). We have discovered a new family of class of acyclic nucleoside phosphonates based on a 4- phosphono-but-2-en-1-yl skeleton, with the double bond having trans stereochemistry, with remarkable nanomolar (nM) antiviral activity against various DNA viruses [EC50 (uM) 0.02 (HCMV), 0.2 (HSV-1), 0.007 (VZV), 0.08 (hAdV-B7), 0.05 (VV) and 0.03 (VV TK-)] with few toxicity. The optimization of the established synthesis route was achieved. By changing the starting point of the synthesis route and by incorporating new protocols the overall reaction yield ranged from 3% (9 steps) to 13% (9 steps), which allows us to prepare gram-scale of LAVR-289, which is required to fund preclinical studies. Additional investigations were then performed to examine other fundamental properties such as metabolic stability, logP, and cytotoxicity of our compound series. Chemical optimized synthesis of LAVR-289 and its biological data will be presented. Supported by the Région Centre Val de Loire (APR-IR Finals), RTR FéRi2 and DGA/AID (RAPID Denalpovir).

153V. Synthesis of cyclic dinucleotides as modulators of STING, a pivotal protein in immunity and antiviral diseases

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STING protein is a unique and pivotal protein of cGAS-STING signaling pathway; its modulation is involved in immunity and STING is considered as a new attractive target to treat infections and cancers. The cyclic dinucleotide (CDN) 2′,3′-GMP-AMP (cGAMP) is the endogenous agonist of STING with known antiviral activities and has served as lead for new CDNs development, such as ADU-S100. In fact, main limitations of cGAMP are inherent to its physical properties e.g. instability regarding hydrolases and charged linkages. Neutral cGAMP analogues that feature better cellular penetrability and resistance facing hydrolysis are still needed. Herein, we report the design and synthesis of two cGAMP analogues with a triazole and an unsaturated chain as new 3′,3′-internucleotide linkages. The convergent synthesis involves as key-step (1) a Cu(I)-catalyzed azide-alkyne cycloaddition and (2) a macrocylisation via a ruthenium-catalyzed ring-closing metathesis. The nucleobases were introduced under Vorbrüggen conditions affording original dimeric-like CDNs analogues. All synthesized compounds were evaluated to determine their activity as STING agonist or antagonist by measuring type I interferon induced secretion IP-10 in macrophages; some of them displayed an interesting biological activity which will be presented.

Supported by European Regional Development Fund (FEDER) and Region Centre Val de Loire for the financial support of EUROFERI program. J. Magand thanks MESRI and UO for PhD salary. A.C. Ojeda-Porras thanks FEDER for her salary.

154V. A Large-scale Drug Repositioning Survey Identifies Clofazimine that Broadly Inhibits Coronaviruses Including SARS-CoV-2

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The collective scientific understanding of COVID-19 has evolved rapidly since its emergence, from recognition of the causative virus to development of multiple vaccines within the span of a year. Repurposing of known drugs could substantially accelerate the deployment of new therapies for COVID-19. Here, we profiled a library of drugs encompassing approximately 12,000 clinical-stage or US FDA-approved small molecules to identify candidate therapeutic drugs for COVID-19. We report the identification of thirteen drugs to harbour effective concentrations commensurate with probable achievable therapeutic doses in patients. In particular, we show that clofazimine, an anti-leprosy drug with a favourable safety profile, possesses inhibitory activity against several coronaviruses. Clofazimine inhibits cell fusion mediated by the viral spike glycoprotein, as well as activity of the viral helicase. Prophylactic or therapeutic administration of clofazimine in a hamster model of SARS-CoV-2 pathogenesis led to reduced viral loads in the lung and viral shedding in faeces, and also alleviated the inflammation associated with viral infection. Combinations of clofazimine and remdesivir exhibited antiviral synergy *in vitro* and *in vivo*, and restricted viral shedding from the upper respiratory tract. Clofazimine, which is orally bioavailable and comparatively cheap to manufacture, is an attractive clinical candidate for the treatment of outpatients, particularly in contexts in which costs are an important factor or specialized medical facilities are limited. Our data provide evidence that clofazimine may have a role in the control of the current pandemic and—possibly more importantly—in dealing with coronavirus diseases that may emerge in the future.

155V. Viral Two-Metal Ion Dependent Enzymes as Antiviral Targets

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Many pathogenic viruses encode essential two-metal ion-dependent (TMID) enzymes that are responsible for RNA and DNA processing reactions. TMID enzymes are structurally related proteins that share common active sites containing conserved carboxylates predicted to coordinate divalent cations essential for catalysis. Drugs that target TMID enzymes, such as HIV integrase and influenza endoribonuclease (PA), have been successfully developed for clinical use; however,

this strategy has not been widely applied for other virus families. We have focused on TMID enzymes encoded by Human Herpesviruses (HHVs) and Coronaviruses (CoVs). HHV TMID enzymes include viral alkaline nuclease (AN), proofreading exonuclease of the viral polymerase (PolExo) and viral terminase. Interestingly, CoVs also encode an essential TMID enzyme, nsp14-ExoN, an exoribonuclease required for proof reading during RNA replication. We have generated a focused screening library comprised of over 200 compounds based on chemotypes designed for their ability to engage the two-metal binding motif. We have identified five leads with potent antiviral activity against HSV, CMV and HHV-6B with EC50s less than 100nM. The most potent leads can inhibit two of the viral TMID nucleases, PolExo and AN, suggesting that it may be possible to develop dual-targeting agents which can inhibit multiple viral enzymes with a single agent. Inhibition of two or more essential enzymes is expected to generate synergistic inhibition and reduce resistance, allowing treatment at significantly lower doses. Two leads have been identified that inhibited CoV nsp14-ExoN activity, physically engaged with the protein in a thermostability assay, and exhibited antiviral activity in cell culture.

156. AI-Derived Antibodies are novel, diverse and pharmacologically active against multiple SARS-CoV-2 strains

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We have developed an AI-generated antibody library platform utilizing a Generative Adversarial Network (GAN) that generates novel sequences which mimic natural human response, as well as biasing toward diversity and developability features. The resulting Humanoid Antibody Library™ (HAL™) was successfully screened to obtain a panel of novel, diverse and pharmacologically active human monoclonal antibodies against SARS-CoV-2. These first-generation antibodies, without the need of affinity maturation, bind to the SARS-CoV-2 spike protein with good specificity and affinity, block the spike: human ACE2 receptor interaction and neutralize SARS-CoV-2 viral infectivity across several strains.

157V. Oral Heat Shock Protein 90 (Hsp90) Inhibitor SNX-5422 Attenuates SARS-CoV-2 Replication And Dampens Inflammation In Airway Cells

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Newly emerging viral infections pose a significant threat to global health and economy. An important example is the ongoing COVID-19 pandemic, which has claimed >5 million lives worldwide. Due to the unpredictable nature of these viruses, development of therapeutics upon viral emergence is often challenging and time consuming, resulting in failure to achieve immediate treatment. Being prepared with an approved therapeutic with broad-spectrum antiviral potency against multiple viruses can rapidly contain a viral outbreak and prevent its transition to a global pandemic. Cellular chaperone protein, Hsp90, is a viral host dependency factor. We and other groups have previously identified an orally bioavailable Hsp90 inhibitor, SNX-5422, currently in trials for cancer therapy, to have antiviral effect against HIV and CHIKV. Here, we tested the broad-spectrum function of SNX-5422, by investigating its efficacy in suppressing SARS-CoV-2 replication. Our data indicate that SNX-5422 treatment of SARS-CoV-2-infected Vero E6 and human lung epithelial (Calu-3) cells attenuated intracellular viral nucleoprotein expression in a dose dependent manner. SNX-5422 also reduced cell-free SARS-CoV-2 genomic copies (IC_{50}:0.2μM, both cell types) and cell-free infectious viral titers (Vero E6 IC_{50}: 0.38μM Vero E6 and Calu-3 IC_{50}: 0.4μM). SNX-5422 treatment of human primary airway epithelial cells dampened expression of pro-inflammatory genes such as *CCL20*, *CXCL1*, *CXCL3*, *CXCL5* and *CXCL6*, previously associated with poor SARS-CoV-2 disease outcomes. Additionally, this drug interrupted expression of SARS-CoV-2 host factors, including *MMP9*, *SERPINE1*, *BUB1B*, *BUB1*, *CENPF* and *ID1*. Development of SNX-5422 as a broad-spectrum antiviral therapeutic will improve pandemic preparedness for future emerging viruses.

158. Oral Pharmacokinetics and Antiviral Activity of 1-O-alkyl-2-O-aryl-sn-glyceryl-3-P-RVn Compounds Predicts Broad Spectrum Efficacy against RNA viruses

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Effective broad spectrum antiviral drugs for treatment and prevention of contemporary and emerging RNA virus infections are urgently needed. Remdesivir has antiviral activity against novel emerging viruses but intravenous administration confines its utility to hospitalized patients. To create oral analogs of remdesivir nucleoside (RVn), we synthesized several glyceryl ether esters of GS-441524 5'-monophosphate, including 1-O-octadecyl-2-O-benzyl-sn-glyceryl ester (ODBG-P-RVn – V2043) (Fig A). V2043 shows low nanomolar inhibitory activity against SARS CoV-2 in multiple cell types and is orally bioavailable in Syrian Hamsters (Antimicrob. Agents Chemother., 2021, §2, e01155-21).

Pharmacokinetic studies of oral V2043 in Syrian Hamsters at 30mg/kg or 50mg/kg were well tolerated and resulted in 12-hour post-administration plasma levels of 4.01ug/ml (±0.73 SD) and 4.55ug/ml (±2.2 SD) respectively (Fig. B). These plasma levels were well above the V2043 EC90s for multiple contemporary and emerging viruses, including SARS-CoV-2 (SCoV2), Ebola virus (EBOV), Nipah virus Bangladesh (Ni-V-B) (bioRxiv 2021.08.06.455494), Respiratory syncytial virus (RSV) and Dengue virus 2 (DENV2), assayed in various cell types (Fig C). Modifications of the long-chain alkyl group (R1), and a 3-fluoro-4-methoxy-substitution on the benzyl group (R2) produced V2051 and V2053 (Fig A), which demonstrated 2-3 fold improved antiviral potency compared to V2043 against both SCoV2 and DENV2 in pluripotent stem cell-derived lung cells and Huh7.5 cells respectively (Fig D). Collectively, our data support the development of lipid prodrugs of RVn monophosphate as potent oral antivirals that could be used to treat many RNA virus infections of public health importance including SARS-CoV-2, RSV, Nipah, Ebola and Dengue infections.

159V. General lipoperoxidators do not target virion lipids specifically

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Emerging viruses are a public health threat. Common viral structures, like virion envelopes, have been proposed as target for broadly-active antiviral drugs. Lipoperoxidators specifically have been proposed to target metabolically inactive enveloped virions without damaging metabolically active cells. However, virion envelopes are protein-rich, which may actually impede the lipoperoxidation chain reaction. Indeed, we have shown that virions are not particularly more sensitive than cells to chemical lipoperoxidators. We now show that whereas losses in cell viability or viral infectivity triggered by the lipid soluble peroxidator AMVN are both highly correlated with increases in lipoperoxidation(r^2> 0.9), the water soluble peroxidator AAPH induced losses in cell viability and viral infectivity at lower concentrations than lipid peroxidation. AAPH thus also acts by lipoperoxidation-independent mechanisms. Antioxidants only partly protected against loss of viral infectivity (< 5- fold), and the carrier BSA alone also protected against both peroxidators. The apparent protection thus likely results from peroxidator quenching. There was no correlation between the effects of antioxidants on viral infectivity or virion lipoperoxidation induced by AAPH. Virions incubated in atmospheric oxidative conditions suffered similar infectivity losses as chemically peroxidated virions. However, no virion lipid peroxidation was detected under atmospheric conditions and water soluble vitamin C and BSA were protective, pointing to peroxidative damage mainly to non-lipid virion components. Lipoperoxidation is thus not a generic approach to specifically inhibit infectivity of enveloped viruses and the effects of peroxidators on virion infectivity are not restricted to lipoperoxidation only.

160V. Characterization of the susceptibility of specific SARS-CoV-2 nsp12 substitutions to remdesivir using a coronavirus reverse genetic system

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Remdesivir (RDV, VEKLURY®), a nucleotide analog inhibitor of coronavirus replication, is the first FDA approved direct-acting antiviral for treatment of SARS-CoV-2 infection. Characterization of the RDV resistance profile is essential for understanding the potential impact of drug resistance in RDV-treated patients. RNA-dependent RNA polymerase (non-structural protein 12; nsp12) substitutions corresponding to V557L and F480L in SARS-CoV-2 emerged following the in vitro selection of murine hepatitis virus (MHV) with increasing concentrations of RDV parent nucleoside GS-441524. Similar selection conducted with SARS-CoV-2 resulted in the emergence of several nsp12 variants that differed from those identified in MHV. A97V and P323L were identified as prevalent substitutions in an analysis of approximately 5 million clinical isolate nsp12 sequences. Additionally, D484Y emerged in an immunocompromised patient following RDV treatment. Each substitution was individually engineered into a recombinant luciferase reporter WA1 strain of SARS-CoV-2 (rSARS-CoV-2) using a reverse genetics rescue system and their susceptibility to RDV was evaluated in A549/hACE2 cells. The majority of profiled substitutions were associated with <2-fold increase in RDV EC_{50} relative to the reference strain, with the exception of the *in vitro* GS-441524-selected substitutions, which individually resulted in 2.5- to 3.5-fold increase in RDV EC_{50} values compared to WA1 rSARS-CoV-2. Further phenotypic characterization of nsp12 substitution combinations observed during GS-441524 resistance selection is currently in progress and will be reported. The mild changes in RDV susceptibility observed with the evaluated nsp12 substitutions suggest a high resistance barrier for RDV in SARS-CoV-2.

161V. Adaptation of Heartland virus in AG129 mice leads to severe disease that can be effectively treated with the ribonucleoside analog, EIDD-2749

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Heartland virus (HRTV) is an emerging tick-borne bandavirus that causes fever, lethargy, headaches, muscle aches, diarrhea, nausea, thrombocytopenia and leukopenia in humans. The virus, transmitted by the lone star tick (*Amblyomma americanum*), was first isolated in 2009 from patients in Missouri. There have been over fifty cases diagnosed by the Centers for Disease Control and Prevention, with four resulting in death. Thus far, ten states have reported HRTV infections, however the tick vector has a range spanning much of the eastern USA and wildlife surveillance supports this broad geographic range. There are no vaccines or approved therapies available to prevent or treat HRTV disease. Until now, a lethal animal model had only been described in 21-day-old AG129 IFN-α/β and γ receptor deficient mice, severely limiting the utility of the model for vaccine development. Here, we describe the genetic adaptation, natural history of disease, and pathogenesis of a mouse-adapted HRTV (MA-HRTV) that is uniformly lethal in 7-week-old AG129 mice. We utilized this new model to assess the promising ribonucleoside analog antiviral discovered at Emory University and developed by Drug Innovation Ventures at Emory (DRIVE), EIDD-2749. Importantly, we show that once-daily dosing of EIDD-2749 initiated after the onset of symptoms provided complete protection of mice against a lethal MA-HRTV challenge dose and significantly reduced or eliminated viral titers in blood and tissues. Studies to further characterize MA-HRTV and assess the spectrum of EIDD-2749's antiviral activity against bunyaviruses are ongoing.

Supported by the NIH (HHSN272201500008C, HHSN272201700041I, 75N93019D00021) and the Department of Defense (HDTRA1-15-C-0075).

162V. Development of N-Substituted Furopyrroles as EBOV and MARV Anti-filoviral Glycoprotein Inhibitors

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Ebola virus (EBOV) and Marburg virus (MARV), members of the *Filoviridae* family, are considered Category A biowarfare agents because of their stabilities in aerosolized form, high fatality rates, and lack of effective therapies for treatment in humans. The 2014-2016 Ebola epidemic in West Africa, which was the largest recorded outbreak; the recent 2018 epidemic in the Democratic Republic of Congo, which is the tenth outbreak since 1976 and the second-biggest Ebola epidemic; and a new outbreak in Guinea, reported in February of 2021, underscore the persistent threat of Ebola epidemics and the need for drug discovery and development efforts to produce effective treatments. Filoviral glycoprotein (GP), known to facilitate viral infection, has proven to be a drug-targetable site with recent FDA-approved drug Inmazeb, a cocktail of three anti-EBOV GP monoclonal antibodies. We have discovered a series of *N*-substituted furopyrroles that display excellent potency against both MARV and EBOV when tested in a VSV-based pseudovirus assay. Selectivity and potency of these viral entry inhibitors were improved by structural modifications on the scaffold, which included optimization of the heterocyclic core and the substituents on the amide portion of the molecules. The best performing inhibitors resulted in nanomolar EC_{50} values with selectivity index values higher than 100. These compounds were validated in infectious assays and found to be potent inhibitors of EBOV and MARV. Our lead compounds display excellent *in vitro* metabolic stability and druglike properties and have potential to be developed as a new class of antiviral drugs.

163V. Exploring the morphology of the host cell to open new possibilities in drug repurposing

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The continued threat by emerging viruses to humankind has highlighted the need for new approaches to identify novel or repurposed drugs. Currently, the majority of antiviral screening methods evaluate the drug activity on a given virus and neglect the host mechanisms that the virus hijacks. To include host cell morphology changes in antiviral screening we combined morphological profiling using the Cell Painting assay with antibody-detection of viral infection and screened 5144 compounds in the SPECS drug repurposing library. SARS-CoV-2 infection induced a virus-specific phenotypic signature in Vero E6 cells, which was reversed by assay controls such as remdesivir. Our unbiased host-focused approach identified additional ~70 compounds that were able to revert SARS-CoV-2 infected host cells towards a healthy cell phenotype. To further deepen our understanding on the host response during infection, we quantified the subcellular localization and expression of 602 host proteins using antibodies from the Human Protein Atlas. We identified phenotypic responses to SARS-CoV-2 infection in 97 proteins. Finally, to broaden the paradigm of how antiviral therapies are identified we aim to combine these host focused screening approaches. Our preliminary analyses have identified 3 potential drug targets linked to 3 compounds that reverse the virus-specific phenotype, which all represent novel drug repurposing candidates against SARS-CoV-2. Taken together, these findings illustrate a new host-centric approach to discovery antivirals and could be applied for other emerging or re-emerging viruses.

164V. Integrated pipeline using ANCHOR™ tagged autofluorescent viruses for accelerated discovery of antiviral molecules

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The end of 2019 saw the emergence of a new coronavirus responsible for pneumonia in Humans: SARS-CoV-2. 100 years after the Spanish flu of 1918, this contagious and potentially fatal virus circulated rapidly throughout the world, causing a pandemic situation that will mark history. Antiviral discovery and validation should therefore be done as a continuous process and all viruses should be considered as a potential threat. We have developed a new visualization technique called ANCHORTM that allows us to visualize directly in real time in living cells infection, localization, replication and cell-to-cell spread of any kind of viruses having a dsDNA phase. This technology has been successfully used to image different kind of viruses, including herpesviruses, adenovirus, poxviruses and retroviruses. Combined with automatic pipetting robot and high content microscopy, the technology allows the screening of a large number of molecules or conditions very rapidly and efficiently without any fixation, extraction or reagent. Based on our expertise, we have also developed image based screening pipeline for RNA viruses such as RSV, influenza and SARS-CoV-2. With a collection of around 30 viruses impacting human, animal and biodefense market, we propose one of the most integrated antiviral screening pipeline so far, from early discovery to animal testing in A3 animal facilities. Two antiviral molecules discovered in the lab will be presented, the first one impacting the animal market and the second one funded by a large grant from the French ministry of Defence.

165. Combinations of directly acting and host-targeting antiviral drugs confer synergistic suppression of SARS-CoV-2 infection

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Most monotherapies tested clinically against COVID-19 yielded disappointing results. Since combination antiviral drug treatments are the norm for chronic RNA virus infections, and since our past work demonstrated that combinations of approved drugs can synergistically inhibit filoviruses (e.g., Ebola) and arenaviruses (e.g., Lassa), we are further exploring the development of self-administered (oral, inhaled) drug cocktails as a first line of defense against viral pathogens. Here, we demonstrate the utility of combining approved and investigational host-targeting antiviral (HTA) and directly acting antiviral (DAA) drugs to suppress SARS-CoV-2 infection. Six approved HTAs and/or DAAs with broad spectrum antiviral activity through suppression of virus entry were interrogated for suppression of infection of Vero E6 monkey kidney cells and Calu 3 human lung cells by vesicular stomatitis virus (VSV) reporter viruses pseudotyped with the SARS-CoV-2 Spike protein. While all six entry inhibitors suppressed VSV-Spike infection of Vero E6 cells, only three suppressed VSV-Spike infection of Calu 3 cells. Among these we tested combinations of the TMPRSS2 inhibitors camostat and avoralstat with the SARS-CoV-2 polymerase inhibitor molnupiravir. In conjunction with molnupiravir, both TMPRSS2 inhibitors conferred synergistic suppression of authentic SARS-CoV-2 infection in 293T cells expressing ACE2 and TMPRSS2 as well as in Calu 3 cells. Computational analysis using pharmacokinetic, pharmacodynamic, and viral dynamic models is now assessing if these combinations have potential clinical efficacy in humans. The data support further development of DAA and HTA combinations as front-line countermeasures for SARS-CoV-2 and variants of concern that can be taken orally in outpatient settings.

167. Broad-Spectrum In Vitro Antiviral Activity of ODBG-P-RVn: An Orally-Available, Lipid-Modified Monophosphate Prodrug of Remdesivir Parent Nucleoside (GS-441524)

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The necessity for intravenous administration of remdesivir confines its utility for treatment of coronavirus disease 2019 (COVID-19) to hospitalized patients. We evaluated the broad-spectrum antiviral activity of ODBG-P-RVn, an orally available, lipid-modified monophosphate prodrug of the remdesivir parent nucleoside (GS-441524), against viruses that cause diseases of human public health concern, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). ODBG-PRVn showed 20-fold greater antiviral activity than GS-441524 and had activity nearly equivalent to that of remdesivir in primary-like human small airway epithelial cells. Our results warrant in vivo efficacy evaluation of ODBG-P-RVn.

185. Prophylactic Efficacy of Intranasal Administration of IgY Antibodies Targeting Receptor Binding Domain Against SARS-Cov-2 Infection in a Transgenic Mouse Model

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The development of new treatments against COVI D-19 is a time consuming and expensive process specially for resource limited countries. Immunoglobulin Y (IgY) is the primary antibody in oviparous animals and have drawn attention as potential alternatives for passive immunization. In this study, we report the generation, characterization and antiviral activity of IgY-Abs targeting SARS-CoV-2 RBD. Lohmann laying hens were injected with recombinant SARS- CoV-2 RBD protein; IgY-Abs were extracted from the eggs and characterized using SDS-PAGE. The specificity was confirmed by Western blotting and the titre was assessed using ELISA. The antiviral activity was evaluated using microneutralization assay and plaque reduction neutralization test showing a ND_{50} <0.05µg/ml and an IC_{50} of 4.44 µg/ml; respectively. In transgenic mouse model, intranasal administration of the generated IgY-Abs, two hours before infection, showed reduced viral replication in lungs as compared to control groups that were received non-specific IgY-Abs. The antiviral activity of the IgY-Abs was also reflected in significant reduction in the weight gain of the treated group compared to the control group. Histopathological examination of the lungs showed reduced perivascular and peribranchial inflammatory cell infiltration, hemorrhage, and edema in the treated group. Our data provide first evidence for the potential use of the generated IgY antibodies as a prophylactic vaccine against SARS-CoV-2. Clinical trials are needed to evaluate anti-RBD IgY antibodies as protective biological product against SARS-CoV-2. The results provide a proof of concept for the use of IgY-Abs for the prophylaxis of SARS-CoV-2.

186. 1-O-Alkyl-2-O-substituted-sn-glyceryl Esters of GS-441524 5′-monophosphate: Synthesis and Antiviral Activity against SARS-CoV-2

James Beadle, Ph.D.[1], Aaron Carlin, M.D.[1], Nadejda Valiaeva, Ph.D.[1], Alex Clark, Ph.D.[1], William Bray, Ph.D.[1], Aaron Garretson, Ph.D.[1], Xing-Quan Zhang, Ph.D.[1], Joyce Murphy, B.S.[1], Robert Schooley, M.D.[1], Karl Hostetler, M.D.[1]

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Effective antiviral drugs for treatment and prevention of SARS CoV-2 infection are urgently needed. Early intervention with antivirals, including intravenous remdesivir (RDV), has been shown to reduce hospitalization and severe disease due to COVID-19. Devising a convenient, orally bioavailable RDV analog might facilitate early administration to

non-hospitalized COVID-19 patients. To create oral remdesivir analogs, we synthesized glyceryl ether esters of GS-441524 5′-monophosphate (Figure 1), incorporating long-chain alkyl groups (R_1), and various substitutions (R_2) that resemble lysophospholipids. The prodrugs are stable in plasma, and converted intracellularly to the active triphosphate metabolite. We reported that compound 1, the 1-*O*-octadecyl-2-*O*-benzyl-*sn*-glyceryl ester (ODBG-P-RVn), inhibits SARS CoV-2 replication in Huh 7.5 cells (EC_{50} = 138 nM) and is orally bioavailable in Syrian Hamsters (*Antimicrob. Agents Chemother.*, 2021, **65**, e01155-21). To evaluate other promoieties for improved efficacy, oral bioavailability and lung-targeting, we synthesized additional 1-*O*-alkyl-2-*O*-substituted glyceryl analogs, including various alkyl, cycloalkyl, and substituted benzyl groups and assessed their anti-SARS CoV-2 activity in Huh 7.5 cells. Among the new analogs, the 2-*O*-octyl substitution (compound 2) was the least potent SARS CoV-2 inhibitor (EC_{50} = 710 nM) while compound 3, with a 3-fluoro-4-methoxy-substituted benzyl group, showed 2.5-fold improved antiviral potency (EC_{50} = 54 nM) vs 1 with unsubstituted benzyl. The in vitro selectivity index of 3 was also improved vs 1. Similar results were noted for Calu-3 cells. Collectively, our data support the development of RVn lipid prodrugs as potent oral antivirals that could be used early in the course of COVID-19 to prevent serious disease requiring hospitalization.

188. A structural model of the SARS-CoV-2 replication and transcription complex

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While structures of the individual components that make up the SARS-CoV-2 replication and transcription complex (RTC) are known, how they assemble into a functioning complex has not yet been determined. Using molecular modeling tools, we have generated a model of the RTC which centers around hexameric nsp15. The resulting superstructure is composed of nine distinct proteins and 62 subunits. In brief, the nsp15 hexamer is capped on two faces by trimers of nsp14/nsp16/$(nsp10)_2$. A conformational change of nsp14, necessary to facilitate binding to nsp15, then recruits six nsp12/nsp7/$(nsp8)_2$ polymerase subunits. To this, six nsp13 subunits are arranged unevenly around the complex. This assembly of the key proteins associated with viral genome replication positions the nsp14 exonuclease and nsp15 endonuclease sites in line with the dsRNA exiting the nsp12 polymerase site. Nsp10 acts to separate the RNA strands, directing the nascent strand to the three sites responsible for mRNA capping: nsp12 NiRAN, nsp14 *N7*-methyltransferase and nsp16 2′*O*-methyltransferase. Finally, the N-protein binds between a pair of nsp13 subunits, positioning the TRS-L RNA sequence above the polymerase active site, a state that is critical for transcription. Overall, the model presents a unifying picture of the diverse set of processes involved in coronavirus genome replication and transcription.

189. In silico discovery of novel potential RNA-binding small molecules against SARS-CoV-2

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In recent years, RNA structure elements have become one of the most attractive emerging targets for antiviral drug discovery. Viral genomes contain some highly conserved RNA elements that play a critical role in gene regulation and viral replication. Programmed ribosomal frameshifting (PRF) is one of the strategies commonly used by some RNA viruses to regulate viral protein expression, making the PRF an attractive therapeutic target for antiviral drug development. Through an *in-silico* structure-based approach, we have recently identified a new family of drug-like small molecules able to target the PRF of SARS-COV-2, showing antiviral activity against multiple variants. Using the cryo-EM crystal structure of the SARS-COV-2 PRF, we have identified a potential binding site in the stem 1- J3/2 pseudoknots region. We hypothesized that small molecules could directly target this region, causing conformational alteration in the RNA frameshifting structure and consequently blocking the viral replication. To test our hypotheses, we virtually screened a library of 44520 commercially available RNA-binding compounds against the identified binding pocket using an established workflow of docking software with increasing accuracy. At the end of our workflow, 20 hits were selected, purchased, and evaluated in antiviral assays. Among them, 5 compounds could inhibit the virus replication with an IC_{50} in the low micromolar range with no evidence of toxicity at the tested doses. These small molecules represent a promising class of compounds, which show more favourable drug-like properties than other RNA-binding molecules, potentially engaging RNA through a combination of shape complementarity and hydrogen bonding interactions

190. Natural-derived inhibitors of SARS-CoV-2 nsp13 unwinding and ATPase activities a starting scaffold to develop antiviral agents

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SARS-CoV-2 infection is still spreading worldwide enlightening the urgent need for new antiviral therapies to complement the approved vaccine preparations. SARS-CoV-2 nps13 helicase is a validated drug target as a key component of the viral replication complex. NSP13 possess two associated activities: RNA unwinding and 5'-triphosphatase. In the search of SARS-CoV-2 direct antiviral agents, we established biochemical assays for both SARS-CoV-2 nps13-associated enzyme activities and screened both in silico and in vitro a small in-house library of natural compounds. Myricetin, Quercetin, Kaempferol and Flavanone inhibit the SARS-CoV-2 nps13 unwinding activity at nanomolar concentrations, while Licoflavone C blocks both SARS-CoV-2 nps13 activities at micromolar concentrations. Mode of actions studies showed that all compounds are nsp13 non-competitive inhibitors versus ATP, while computational studies suggested that they can bind both nucleotide and 5'-RNA nsp13 binding sites, with Licoflavone C showing a unique pattern of interaction with nsp13 amino acid residues. The inhibitor binding mode offers good possibilities for scaffold optimization. Overall, we report for the first time some natural compounds as selective inhibitors of SARS-CoV-2 nps13 helicase with nanomolar activity, which represent a good starting point for the achievement of effective antiviral drugs.

191. Development of a Rapid MoA-based Potency Bioassay using Virus-like Particles for Assessment of SARS-CoV-2 Entry Inhibitors

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Vaccines and neutralizing monoclonal antibodies (mAbs) have proven effective in reducing COVID-19 transmission, morbidity, and mortality. Nonetheless, the emergence of SARS-CoV-2 variants has necessitated frequent reevaluation of existing vaccines and mAbs and the development of new therapies to combat potential resistance. One bottleneck for SARS-CoV-2 neutralizing mAb and vaccine programs is access to safe, rapid, and biologically-relevant potency assays to measure neutralization of different SARS-CoV-2 variants. To meet this need, we have developed a quantitative bioassay platform utilizing a HiBiT/LgBiT split luciferase system. HiBiT-tagged virus-like particles are pseudotyped (HiBiT-PsVLPs) with SARS-CoV-2 Spike (S) protein. Entry of these SARS-CoV-2 S HiBiT-PsVLPs into LgBiT-expressing target cells results in HiBiT/LgBiT complementation and formation of a functional NanoLuc luciferase reporter. Unlike live virus or pseudoviruses, SARS-CoV-2 S HiBiT-PsVLPs are non-replicating and lack viral nucleic acid, allowing for work at reduced biosafety levels. Luminescent assay signal is not dependent on reporter gene expression, enabling a <5-hour assay workflow from setup to readout. SARS-CoV-2 S HiBiT-PsVLP entry is ACE2- and TMPRSS2-dependent and is inhibited by neutralizing anti-SARS-CoV-2 mAbs, serum from vaccinated donors, and small-molecule TMPRSS2 inhibitors (camostat mesylate and nafamostat mesylate). Importantly, we show that HiBiT-PsVLPs bearing S proteins from SARS-CoV-2 variants (Alpha, Beta, Gamma, Delta, etc.) exhibit differing sensitivity to research-grade analogues of therapeutic anti-SARS-CoV-2 neutralizing mAbs (casirivimab, imdevimab, bamlanivimab, and etesevimab). Overall, these data demonstrate that the HiBiT-PsVLP assay platform is suitable for measuring activity of SARS-CoV-2 entry inhibitors with the flexibility needed to address SARS-CoV-2 variants and other viruses with pandemic potential.

192V. Comparative infectivity of emerging SARS-CoV-2 variants in human airway epithelial cell cultures

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Incubation of human airway epithelial cells (HAECs) from small airway origin (HsAEC), with the same amount of infectious virus units, resulted in a productive infection in only 17% of the cultures incubated with USA-WA-1 isolates while 42%, 92%, and 75% of the cultures showed a productive infection when incubated with BavPat1, B.1.1.7, and B.1.351 strains respectively (n=12). Alpha and beta variants replicated more efficiently during the first 6 days of the experiment than the ancestral strains while no difference in relative virion infectivity (i.e. the ratio of the number infectious particles over the number of viral RNA) was observed. A similar observation was made in HAECs of tracheal origin (HtAEC). While all cultures could be infected by either the B.1.1.7 or B.1.351 variant, only 50% of cultures could be infected with either USA-WA-1 or BavPat1 isolates (n=6). The replication kinetics of two VoC was better than that of the ancestors but again no disparity in the relative virion infectivity was observed. A panel of cytokines linked to COVID-19 in humans including IP-10, IL-6, IL-10, TNF-α, and TFN-ɣ was monitored up to day 8 p.i.. While there was an upregulation of IP-10 in the B.1.351-infected group both in HsAEC and HtAEC, the overall discrepancy in cytokine expression between variants was limited. Taken together, our findings demonstrate that the alpha and beta variants infect and replicate more efficiently in HAECs when compared with the ancestral lineages.

193. A New Mouse Model of SARS-Cov-2 Infection as a Tool for Evaluating COVID-19 Vaccines and Therapeutics

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Infection with SARS-CoV-2 causes coronavirus disease 2019 (COVID-19). To understand the pathophysiology of COVID-19 and to test potential vaccines and therapeutics, appropriate animal models that mimic the biology of human SARS-CoV-2 infection are urgently needed. We developed a mouse model in which human ACE2 (hACE2) is expressed by cells of the upper and lower respiratory tract after inhalation of a modified adeno-associated virus (AAV) (AAV-hACE2). This approach enables the rapid production of large cohorts of mice and has the advantage of being applicable to wild-type and mutant mouse colonies, independently of age and sex. Moreover, because AAV vectors are only weakly immunogenic and cytotoxic, the system allows for the prolonged expression of hACE2. SARS-CoV-2 infection of these hACE2 mice results in progressive weight loss, respiratory pathology and disease that require culling at 7-8 days post-infection (dpi). The lung pathology of these mice resembles that associated with severe COVID-19 in humans and is characterized by diffuse alveolar damage, alveolar replacement with infiltrates of immune cells and fibroblasts, thickened septa and infiltrations by activated macrophages with foamy cytoplasm. Also, diet-induced obese and lean control hACE2 mice, transduced for ACE2 expression using replication-defective adenovirus, were infected with SARS-CoV-2, and monitored for lung pathology, viral titers, and cytokine expression to simulate the situation in high-risk COVID-19 patients. These models were successfully used to test novel therapeutics for COVID-19, including a highly potent bispecific IgG, which has been developed on the basis of two antibodies derived from donors who had recovered from COVID-19.

194. Cyclophilin Inhibitors Enhance Cell-Autonomous Antiviral Immunity to Inhibit Coronavirus Infection

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Cyclophilins (Cyps) are essential host factors for many RNA viruses, including coronaviruses (CoVs). Cyclophilin inhibitors (CypI) have broad antiviral activities, although a clear understanding of their antiviral mechanisms has yet to be elucidated. To address this gap, we sought to characterize the antiviral mechanisms of CypI against HCoV-229E, using the classical CypI cyclosporine A (CsA) as a model. We show that treatment of HCoV-229E-infected A549 lung alveolar epithelial cells with CsA not only inhibits CoV replication, but also induces expression of interferon-stimulated genes (ISGs) with known antiviral activities, such as Mx1. However, CsA treatment did not modulate classical IFN pathways, as demonstrated by a lack of induction in IRF3 and NF-κB activity, measured by luciferase reporter assay. Furthermore, the presence of the JAK inhibitor ruxolitinib, which inhibits IFN signaling and therefore the induction of

classically activated ISGs, affected neither CsA antiviral potency nor induction of Mx1 expression, indicating that these effects are independent of IFN signaling. Mx1 expression is dependent on the transcription factor interferon regulatory factor 1 (IRF1), which regulates the basal expression of a panel of antiviral genes. Interestingly, RNAi-mediated silencing of IRF1 reduced the antiviral potency of CsA and dampened CsA-induced Mx1 expression. We propose a model where CypI treatment induces IRF1-dependent antiviral immunity, thus contributing to its broad spectrum of antiviral activity. While mechanistic characterization is ongoing, these findings provide a novel antiviral paradigm for cyclophilin inhibitors and open perspectives for novel therapeutic approaches against CoVs and other RNA viruses, many of which are currently untreatable.

195. Disruption of Conserved Heparan Sulfate-Dependent Attachment as an Antiviral Strategy for Emerging Coronaviruses

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The SARS-CoV-2 pandemic marks the third emergence of a highly pathogenic coronavirus (CoV) in the 21st century. The diversity of CoVs in bats highlights the likelihood of future spillover into human populations. We aim to develop broadly-acting antivirals that could be rapidly deployed to prevent CoV infection. Most human viruses, including CoVs, initiate attachment to cells through interactions with glycans. Blocking these primary glycan-dependent interactions is a demonstrated approach to inhibit entry of diverse viruses. However, the development of a pan-CoV entry inhibitor with therapeutic potential remains elusive. As proof-of-concept, we show that the natural product epigallocatechin gallate (EGCG) inhibits entry of endemic human CoVs (HCoV-229E, HCoV-OC43) and highly pathogenic emergent and pre-emergent CoVs (SARS-CoV-2, MERS-CoV, bat WIV1-CoV). Our results show that EGCG inhibits CoV attachment by competing with heparan sulfate for virion binding, revealing a conserved role for heparan sulfate proteoglycans (HSPGs) in mediating CoV attachment. We show that heparin pre-treatment inhibits entry and attachment of SARS-CoV-2, WIV1-CoV and, unexpectedly, HCoV-OC43. To further confirm the role of HSPGs in CoV infection, we used CRISPR/Cas9 to knock-out EXT1, a glycosyltransferase required for the biosynthesis of heparan sulfate, and are investigating the impact on infectivity of SARS-CoV-2, WIV1-CoV, HCoV-OC43, and MERS-CoV in susceptible cell lines. Furthermore, we are evaluating the roles of human syndecans and glypicans, the two major families of HSPGs, in CoV entry. These findings are elucidating the roles of HSPGs as broad CoV attachment factors, while informing rational design of pan-CoV attachment inhibitors to enhance our pandemic preparedness.

196. Discovery of Highly Potent Oligonucleotides Targeting SARS-CoV-2

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Background: Oligonucleotides offer a powerful means to prevent and treat SARS-CoV-2 infections. Here, we aimed to identify siRNA's and ASO's from a panel of in silico designed oligonucleotides using an in vitro screening system. Methods: 190 siRNAs and 130 ASOs were designed using in-house bioinformatics, selecting for conserved SARS-CoV-2 target regions and reduced risk of off-target activity. In vitro screening utilized a vector overexpressing luciferase-fused SARS-CoV-2 genome fragments . The antiviral activity of the most potent oligonucleotides was evaluated using A549-hACE-2 cells, infected with nanoluciferase-tagged SARS-CoV-2 virus. Results: We identified 14 siRNAs displaying EC_{50} values < 1.5 nM and maximum inhibition of reporter expression > 70% in the reporter assay. Upon assessment of antiviral activity, we identified two highly effective siRNAs significantly reducing virus replication with inhibition above 90% and EC_{50} values of < 0.5 nM. Additional chemical modifications resulted in highly optimized siRNAs with enhanced antiviral activity and EC_{50} values of ~20 pM for the two lead siRNAs. Similarly, we identified and optimized an ASO with strong antiviral activity (EC_{50} of 68 nM, 78% maximum inhibition). Lead siRNAs and ASOs do not induce cytotoxicity at concentrations up to 500 nM. Based on bioinformatics analysis, the lead siRNAs and ASOs are expected to maintain antiviral activity against SARS-CoV-2 variants of concern and SARS-CoV-1. Conclusions: We developed a platform and identified highly potent oligonucleotides that can be used for therapeutic and preventative treatment of COVID-19. Current work focuses on optimizing the delivery of the lead compounds to lung epithelial cells.

197. An ultrapotent DARPin molecule as a broadly neutralizing inhaled therapeutic against COVID

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SARS-CoV-2 exhibits remarkable adaptability and multiple variants with increased infectivity have emerged, threatening to obsolete the current vaccines and therapeutic antibodies and perpetually prolong the pandemic. Although a number of monoclonal antibody therapeutics have received emergency use authorization and have demonstrated impressive efficacy in patients, they suffer from high production cost, limited global supply and inconvenient route of administration, as well as limited spectrum of neutralization rendering them vulnerable to viral resistance development and often requiring cocktail therapy to remain clinically relevant. As an alternative, we report the engineering an ultra-potent and broadly neutralizing synthetic protein – FSR16m, a homo-trimeric DARPin protein – as a nasally delivered therapeutic for treating SARS-CoV-2 infection. The IC_{50}s of FSR16m against the authentic beta B.1.351 and delta B.1.617.2 variants were 1.76 ng/mL (47 pM) and 2.33 ng/mL (30 pM), respectively. Affinity studies confirmed that FSR16m can efficiently bind a panel of 21 receptor-binding domain (RBD) mutants, including many that are resistant to the antibody therapeutics currently authorized for emergency usage. Intranasally administer FSR16m effectively protected mice infected with the authentic delta B.1.617.2 variant resulting in significantly reduced weight loss and 10-100-fold reduction in viral RNA load in lungs, hearts and nasal washes. The strong neutralization potency, combined with its broad neutralization spectrum, render FSR16m a promising candidate as intranasally delivered therapeutic for treating and preventing SARS-CoV-2 infection.

198. The pathogenesis of COVID-19 depends on SARS-CoV-2 exposure dose and frequency and persists after recovery from clinical infection in a mouse model

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COVID-19 pathogenesis ranges from mild to lethal even among people with similar risks. Antivirals may reduce replication, hospitalization, or lethality, and pathogenesis depends on viral replication and host responses. A robust model is required to test different endpoints. We evaluated the effects of exposure dose and frequency on the clinical, virological, pathological, and molecular outcomes of K18 hACE2 transgenic mice infected with SARS-CoV-2 USA-WA1/2020. Groups of five male and five female mice were infected with 24,000 - 720,000 infectious units by single or triple exposures 24 hours apart. Animals were necropsied daily from day 4, or at euthanasia criteria. Viral infectivity and RNA shedding, weight, and clinical signs were evaluated daily, and viral infectivity, RNA load, and transcriptomics in lungs and nostrils, and lung histopathology, after necropsy. We analyzed the results, and explored COVID-19 determinants and correlates, using a spatially structured computational model. Repeated exposures and higher doses lead to worse outcomes. All animals exposed thrice to either 8,000 or 240,000 infectious units reached euthanasia criteria on seven days, and nine of ten exposed to 240,000, on five. Animals exposed only once reached euthanasia criteria in up to 10 days, and one animal in each of the groups exposed once to 24,000 or 240,000 infectious units recovered from all clinical signs from day eight. Lung transcriptomic changes were subsequent to, and persisted longer than, viral replication and recovered mice had persistent microscopic lung lesions. Resolution of viral replication therefore does not result in prompt resolution of lung pathology.

199. Replication and antiviral activity of MERS and SARS-CoV-2 variants in a highly specialized 3D mucociliary tissue model consisting of normal, human-derived tracheal/bronchial epithelial cells

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In vitro assays are an important early step to determine the potential antiviral activity of novel compounds. The antiviral efficacy of compounds is often evaluated in immortalized cell lines, which offer the advantage of cost effectiveness, ease of use, and reproducibility. However, these monolayer cell lines are not always predictive for cytotoxicity or in vivo efficacy. The EpiAirwayä 3D mucociliary tissue model consisting of normal, human-derived tracheal/bronchial epithelial cells (MatTek) provides a human-relevant tissue model for respiratory disease research. We have developed an advanced antiviral assay to evaluate the efficacy of promising compounds against Middle East Respiratory Syndrome Coronavirus (MER-CoV) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infections by constructing virus growth curves on the EpiAirwayä 3D mucociliary tissue model. Antiviral activity was measured by virus yield reduction assays and cytotoxicity was evaluated microscopically and by MTT colorimetric assay. The test compounds remdesivir and EIDD-1931 were shown to significantly reduce viral replication against six strains of SARS-CoV-2 and MERS-CoV, but ribavirin was not significantly efficacious. Additionally, the EpiAirwayä tissue model was more sensitive for detecting antiviral effects than was observed on some common cell lines (MA-104, A549, HeLa-Ohio, MDCK, and/or Vero cells). The EpiAirwayä human tissue model is a valuable tool for evaluating potential antiviral compounds prior to advancement to in vivo models for respiratory diseases. Future antiviral assays using nasal and small intestine tissue models of human disease are currently being developed.

200. Structural basis of nucleotide recognition by the SARS-CoV-2 RNA dependent RNA polymerase

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The COVID-19 pandemic has claimed millions of lives and decimated the world economy. Understanding the basis of how the causative agent, SARS-CoV-2, replicates its genome will guide the development of therapeutics against this critical process. Integral to replication is the viral RNA-dependent RNA polymerase (RdRp) that acts in concert with a coterie of viral nucleic acid processing enzymes to produce nascent copies of the viral genome and subgenomic-mRNAs, encoding for viral structural proteins. The RdRp is a proven drug target in which two antivirals, remdesivir and molnupiravir, are in clinical use for the treatment of COVID-19. To aid the development of coronaviral RdRp specific nucleotide analogs, we utilized cryo-electron microscopy (cryo-EM) to capture snapshots of the RdRp as its Michaelis complex in the presence of each of the four natural nucleoside triphosphates and the activated form of remdesivir, remdesivir triphosphate (RTP). Our structures, with nominal resolutions ranging from 2.6 Å–3.3 Å, illustrate how the RdRp catalytic motifs discriminate between individual nucleotide bases, prior to their incorporation into the growing nascent RNA. In addition, comparison of the pre-incorporation complexes provides insights to why RTP is incorporated more selectively than its natural ATP counterpart. Furthermore, our investigation identified a novel binding mode of GTP in the nidovirus RdRp-associated nucleotidyl transferase (NiRAN) domain, an essential protein domain that lies N-terminal of the RdRp. Our results provide evidence toward a blueprint of the RdRp in action, aiding structure-based drug design approaches which is a critical endeavor in the hunt for coronavirus therapeutics.

201. A Hypothesis: Covid-induced Micro-clots are Formed Only During Sero-conversion.

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The severe and complex COVID-19 symptoms, often needing hospital care, occur suddenly, late in the virus replication stage of SARS-CoV-2 infection. Hospitalised patients have tissue damage due to micro-clots at multiple sites. Could long-covid, also clinically variable, be a milder form caused by "nano-clots", too small to cause detectable tissue damage? The timing and suddeness of the onset of serious disease needs an explanation. A paper (https://doi.org/10.1016/

S0140-6736(20)31094-1) reported on the first 20 children needing urgent hospital care. No child had any symptoms prior to suddenly becoming seriously ill, only one had a positive PCR test but all had positive SARS-CoV-2 antibody test. That finding indicated to me that this onset occurred at the moment of sero-conversion. An emerging viral moiety, the virion-IgG-antibody complex, has concentrations rapidly increasing, then decreasing. It is known that influenza-IgG-antibody-complex consists of many virions bound together by the antibody. It seems a reasonable step to propose that SARS-2, being a large virus and having many spike proteins, can form larger clumps of virus-IgG-antibody complexes than with influenza, the former being sufficiently large to initiate micro-clots. Therefore, my hypotheses is that the SARS-CoV-2 virion-antibody complex initiates micro-clot formation. Either before or after seroconversion, the complex will be at too low a concentration to initiate micro-clots. To be useful, a hypothesis should be testable, provide explanations and give predictions. My presentation will give examples. I like the way that several apparently separate pieces of a jig-saw puzzle have come together to form a single picture.

202V. Remdesivir retains potent antiviral activity against the SARS-CoV-2 Delta variant and other emergent variants

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Numerous SARS-CoV-2 variants have emerged during the COVID-19 pandemic, including the predominant Delta variant of concern. Remdesivir (RDV, VEKLURY®) is currently the only FDA-approved direct acting antiviral against SARS-CoV-2 infection. Here, we evaluated the in vitro antiviral activity of RDV against the SARS-CoV-2 Delta variant and additional emergent variants by plaque reduction assay (PRA) and nucleoprotein (N) ELISA in A549-ACE2-TMPRSS2 cells. The average RDV EC50 against the Delta variant was 23.7 nM by PRA and 46.2 nM by N protein ELISA, resulting in EC50 fold changes of 0.38 and 0.49, respectively, compared to the WA1 reference (EC50 = 62.1 and 94.4 nM by PRA and ELISA, respectively). RDV EC50 values against eight other clinical variants including Alpha through Lambda isolates exhibited a range of 14.5-101.4 nM by PRA and 45.1-177.3 nM by ELISA (fold EC50 changes of 0.23-1.63 and 0.48-1.88 compared to WA1, respectively). As the Delta variant contains two substitutions (P323L/G671S) in Nsp12, the RNA dependent RNA polymerase, which is the target of RDV, either P323L alone or the P323L/G671S double substitution was introduced into recombinant SARS-CoV-2 NanoLuc WA1 virus and assessed for sensitivity to RDV. The EC50 values for RDV against recombinant P323L and P323L/G671S virus were 71 and 104 nM, respectively (fold change of 0.89 and 1.30 compared to WA1). Taken together, our findings indicate that RDV retains potent antiviral activity against the SARS-CoV-2 Delta and all other tested SARS-CoV-2 variants, consistent with the minimal genetic changes observed in their Nsp12 gene.

203V. Synthetic Host Defense Peptide Inhibits SARS-CoV-2 Replication in vitro

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BACKGROUND: Although myriads of potential antiviral agents have been tested against SARS-CoV-2, only a handful have proven to be effective in clinical trials. Here we characterized the ability of a synthetic host defense peptide to block SARS-CoV-2 replication *in vitro*. METHODS: Cellular toxicity was evaluated in multiple cell lines (MDCK, Vero, VeroE6/TMPRSS2, Calu-3, Caco-2, and Huh-7). Antiviral activity was quantified using authentic SARS-CoV-2 strains (CPE, qRT-PCR, and BCA assays), as well as SARS-CoV-2 spike pseudotyped viruses. ELISA-based binding and time-of-drug addition (TOA) assays were used for target identification. RESULTS: The synthetic host defense peptide showed a favorable toxicity profile, with no cell death at 100 µg/ml, while inhibiting authentic SARS-CoV-2 replication with EC_{50} values ranging from 4.4 to 12 µg/ml and SARS-CoV-2 spike pseudotyped virus entry with an EC_{50} of 5.6 µg/ml. The blocking of SARS-CoV-2 entry was confirmed by the TOA assay and the ability of the synthetic host defense peptide to inhibit binding of the virus receptor binding domain to the ACE2 receptor. Finally, we showed that the synthetic host defense peptide, when combined with soluble ACE2, can synergistically inhibit SARS-CoV-2 entry into susceptible cells. CONCLUSIONS: Novel anti-SARS-CoV-2 strategies are needed, particularly those preventing viral infection. Here we showed that a synthetic host defense peptide safely blocks SARS-CoV-2 replication *in vitro*. Ongoing studies will help complete the pre-clinical characterization of this peptide, which could potentially be used as a prophylactic and/or therapeutic drug against COVID-19.

207V. Immune and Inflammatory Profile in Critically Ill COVID-19 Patients After Recovery

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SARC-CoV2 infection can lead to severe complications. We performed an immunological characterization of SARS-CoV2 infected patients after intensive care unit (ICU) recovery

Adults with confirmed SARS-CoV2 infection (SCoV2+;n=16, median age: 56 years), admitted to a Spanish ICU during 2020 were selected. The immunophenotyping of innate and adaptive immune components was studied on peripheral blood mononuclear cells by multiparametric flow cytometry. Up to 13 soluble pro/anti-inflammatory cytokines using bead-based immunoassay and soluble α-1-thymosine (αThy) by ELISA assay were determined in plasma. SCoV2+ patients were compared to non-SARS-CoV2-infected Healthy Donors (HD;n=18) matched by age and sex On NK cell, SCoV2+ group shows low $CD16^{++}$ subset and high expression of the activation marker CD158b+ and inhibitory receptor NKG2A+ on $CD56^{-}CD16^{+}$ NK cell subset(A). Monocytes from SCoV2+ show increased proportion of intermediate subset and decreased expression of endothelial adhesion and migration markers CD62L+ and CD49d+ on classicals and patrolling subsets respectively(B). No differences were found on cytokine plasma levels, but inversed correlations between activation levels, defined by CD38+DR+ co-expression on CD4 T-cells and soluble IL6; and direct correlations between CD137+ on CD4 T-cells; and IFNγ and ratio CD4/CD8 and IL-10 were observed. NKG2A+ and CD57+ expression on NK cells directly correlated with IFNγ, while CD62L+ on monocytes correlated inversely with soluble IL23(C). Increased αThy levels were observed on SCoV2+ compared to HD; although 6 months after recovery these levels significantly decreased(D). Clinical recovery of severe SARS-CoV2 infection shows differences in activation markers on immunity components and soluble biomarkers that could have future clinical complications.

208V. Immunomodulatory Effect on Dendritic Cells and T-cells of α-1-thymosine (α1Thy) in Response to SARS-CoV2

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This study determines the immunomodulatory effect of α1Thy *in vitro* in response to SARS-CoV2 infection. Healthy donors diagnosed during the last 18 months by SARS-CoV2 infection by PCR+ test, younger than 40 years were selected (n=16) and dendritic cells (DCs) were isolated from peripheral blood mononuclear cells by negative selection and cultured *in vitro* overnight in the absence or presence of α1Thy (50ng/mL). DCs were then characterized by multiparametric flow cytometry using markers for myeloid and plasmacytoid subset discrimitation (mDCs, CD11c+CD123-; pDCs, CD123+CD11c-), activation and co-stimulation (CD40, CD80, TIM-3 and PDL1) and intracellular TNFα production. Autologous T-cells were co-cultured with DCs previously described in the presence or absence of SARS-CoV2 peptides (20µl/ml) during 6 h. Intracellular cytokine production (IL-2, TNFα, IFNγ), cell polyfuncionality and T-cell inmmunophenotyping were also assessed. DCs stimulated with α1Thy showed upregulation of CD40 expression and increased TNFα production, especially on pDCs. Total CD4 T-cells from co-cultures of α1Thy stimulated DCs showed in response to SARS-CoV2 increased levels of CD40L and PD1 (FigA), similar results were observed on CD4 and CD8 T-cells memory subsets, and decreased pro-inflammatory cytokines production was also observed on CD4/CD8 T cells comparing to DCs/T-cell condition in absence of α1Thy pre-stimulation (FigB.). Polyfunctionality measured on T cell was increased in that pre-stimulated condition on total T-cells as well as in their respective CD4/CD8 memory subsets (FigC). Our results suggest that α1Thy could be able to reduce, via DCs modulation, the amount of pro-inflammatory T-cell cytokines and increase the polyfuncionality of T-cells.

209V. SARS-CoV-2 Omicron and Pan-Variant Neutralization Activity of Ensovibep: a DARPin Therapeutic Candidate for Treatment of Covid-19

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Background: The omicron variant of the SARS-CoV-2 virus threatens to alter the COVID-19 pandemic with increased transmissibility, evasion of immunity and potentially reduced efficacy of existing antivirals. Ensovibep, an investigational antiviral biologic, uses 3 distinct DARPin domains to bind to epitopes of the SARS-CoV-2 spike protein trimer receptor binding domain, blocking the interaction with human ACE2. To date, ensovibep has maintained potent neutralization against all variants of concern. Activity against omicron (B.1.1.529) is now described. Methods: The neutralizing potencies of ensovibep, its individual monovalent DARPin domains, and a selection of monoclonal antibodies (mAbs) were assessed using pseudotyped virus assays. The viral glycoprotein was substituted with the SARS-CoV-2 spike protein, carrying the according set of mutations. Live virus assays were performed in an additional test series. Results: In pseudovirus systems containing the fully mutated omicron spike protein, ensovibep showed neutralization at a low IC50 (2.1-3.6 ng/mL) translating to ~1.5-fold potency shift compared to the wildtype virus reference. In contrast, seven out of nine clinically relevant mAbs demonstrated a loss in potency of >40-fold against omicron with IC50 values >100 ng/mL. These studies demonstrate that the tri-specific DARPin design of ensovibep, with cooperative binding, permits potent neutralization across variants, even if an individual monovalent DARPin domain of ensovibep exhibits altered potency. Further results from live virus experiments will be presented. Conclusion: Ensovibep's pan-variant in vitro activity against omicron and other variants of concern supports its continued clinical development. The clinical benefit of ensovibep is being evaluated in the Phase 2b/3 EMPATHY trial.

210V. An interaction of Early pregnancy-associated protein-1 with SARS Cov-2 S protein: Implications in vertical transmission of SARS-2 Cov-2

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A novel severe acute respiratory syndrome (SARS), also known as 2019-nCoV, is a contagious disease caused by SARS-coronavirus (SARS-CoV-2). Viral Spike protein, consists of two functional subunits: S1 and S2. RBD region of S1 subunit binds to the peptidase domain (PD) of human Angiotensin-Converting Enzyme-II (ACE-II) on target/host cell, while the membrane-anchored S2 subunit contains the fusion machinery. ACE2 receptor is expressed in both the upper and lower respiratory tract, myocardium, and gastrointestinal mucosa.SARS-CoV-2 has caused special concerns in pregnant women because both SARS-CoV and MERS-CoV have been found to cause severe complications in pregnant women. It is still unclear the dynamics of transmission of SARS-CoV 2 from the mother to the fetus. Further, the ability of SARS Cov-2 in infecting endothelial cells provides an insight into the possible role of cellular protective factors namely a 90KDa glycoprotein expressedby placental endothelial cells, Epap-1 (early pregnancy-associated protein). Here we report the possible role of Epap-1 in interacting S-protein and regulating ACE2 interaction to understand the mechanisms associated with SARS Cov-2 activities in endothelial cells during early pregnancy. Structural analysis Epap-1 shows a strong interaction with RBD region of SARS Cov-2 spike protein involving residues K417, Y453, Y456, F486, Q498, Y449, N501, Y473, Q474of RBD region with Y61, F287, I302, N303, N305, S334, N465, G467, N468 residues of Epap-1, thus interfering with S protein-ACE2 interaction. The results of the action of Epap-1on S protein-ACE2 suggest the possible role of Epap-1 in protecting SARS Cov-2 infection during early pregnancy.

211V. Identification of Potent Inhibitors of SARS-CoV-2 Exoribonuclease by Fluorescence Polarization Assay

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The emergence of SARS-CoV-2 has triggered a pandemic with devastating consequences to the world. One of the proteins essential to the virus life cycle is nsp14, which is a bifunctional protein that encodes a 3'to 5' exoribonuclease activity in its N-terminus, and a methyl transferase activity in its C-terminus. Nsp14 in complex with the accessory protein nsp10 is involved in a proofreading mechanism that ensures the genetic stability of its massive viral genome, and is associated to the resistance against nucleotide analogs targeting the polymerase nsp12. Because of its key role, nsp14-nsp10 complex constitutes an attractive target for antiviral development. Here we present a fluorescence polarization (FP) assay development to measure the exoribonuclease activity and its inhibition in vitro. The FP method is sensitive, robust, amenable to miniaturization and offers confirmation by visualizing the degradation of the fluorescent RNA in acrylamide gels. We performed a screening of a focused library of 113 metal chelators at 20 and 5 μM compound concentration and IC_{50} measurement of 9 hits showing efficiency at micromolar level. We also tested the focused library in SARS-CoV-2 infected Vero cells and we confirmed 3 hits previously detected in the in vitro screening out of 6 promising inhibitors. In conclusion the FP method proposed is a reliable tool to discover inhibitors of the SARS-CoV-2 exoribonuclease activity and will help to find new antivirals to be used in combination with nucleoside analogs.

212V. PRO-2000 inhibits SARS-CoV-2 replication by interfering with spike-heparin binding

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Here, we report on the anti-coronavirus activity of PRO-2000, a sulfated polyanionic compound that has been under development as a topical microbicide to prevent HIV transmission. In Vero cells infected with the Wuhan, alpha, beta or delta variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), PRO-2000 displayed EC_{50} values of 1.1 μM, 2.7 μM, 1.5 μM and 2.2 μM, respectively, and an average selectivity index (i.e. ratio of cytotoxic versus antiviral concentration) of 44. Virus yield at 5 days p.i. was reduced by 3.8 logs at 4 μM PRO-2000, and its anti-SARS-CoV-2 activity was confirmed in CaCo2 cells, Calu3 cells and A549 cells expressing human ACE2 and TMPRSS2. The entry of SARS-CoV-2 is initiated by interaction of the spike (S) glycoprotein with angiotensin-converting enzyme 2 (ACE2) and heparan sulfate proteoglycans. Surface plasmon resonance studies showed that PRO-2000 binds to the receptor-binding domain (RBD) of S with a K_D of 1.6 nM. Similar K_D values were obtained with the RBDs of the alpha, beta and delta variants (1.6 nM, 2.1 nM and 1.2 nM, respectively). In a neutralization assay, PRO-2000 had no effect on the interaction between the RBD and ACE2. Instead, PRO-2000 was proven to inhibit binding of the RBD to a heparin-coated chip, yielding an IC_{50} of 1.2 nM at 100 nM RBD. Finally, PRO-2000 did not select for resistant viruses after seven passages. To conclude, PRO-2000 has the potential to inhibit a broad range of SARS-CoV-2 variants by blocking the heparin-binding site on the S protein.

214V. Mutations L50F, E166A and L167F in SARS-CoV-2 3CLpro are Selected by a Protease Inhibitor in vitro and are Associated with Resistance

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A SARS-CoV-2 isolate, prototypic Wuhan strain, was passaged in VeroE6 cells in the presence of Compound 1, a covalently bound, reversible and selective 3CLpro inhibitor (EC50 = 1.4 μM; 3CLpro IC50: 8 nM). The starting concentration for the selection was 0.4 μM and was increased to 5 μM at p8 (day 28) and 6 μM at p12 (day 39). Mutations in the 3CLpro were observed at p8 (L50F E166A) and p12 (L50F E166A L167F). Phenotypic analysis of the p12 isolate showed a >3-fold increase in the EC50 values of Compound 1, PF-07321332 (nirmatrelvir), and PF-00835231 while the sensitivity to remdesivir remained unchanged. A cell-based gain-of-function 3CLpro assay confirmed the resistance phenotype. Each of the mutations, alone and in combination, were also introduced into recombinant 3CLpro enzymes. While two of the single mutations (E166A and L167F) provided low-level resistance to the inhibitors in a biochemical enzymatic assay, the triple mutant displayed the highest levels of resistance, ranging from 11- to 80-fold. The mutations were associated with a significant loss of enzymatic 3CLpro activity, suggesting a reduction in viral fitness. Structural biology analysis of the 3CLpro and in silico considerations were used to rationalize this phenotype. To our knowledge, this is the first report of SARS-CoV-2 resistance selection in vitro with 3CLpro inhibitors and provides additional information on the potential role of the E166A substitution as proposed previously (Flynn et al, 2022). The clinical significance of these mutations remains to be determined and the effect on viral replication capacity is being evaluated.

215V. Novel Antiviral Activity of PAD Inhibitors Against Human Beta-coronaviruses HCoV-OC43 and SARS-CoV-2

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The current SARS-CoV-2 pandemic, along with the likelihood that new coronavirus strains will appear in the nearby future, highlights the urgent need to develop new effective antiviral agents. In this scenario, emerging host-targeting antivirals (HTAs), which act on host-cell factors essential for viral replication, are a promising class of antiviral compounds. Herein, we report the discovery of a new class of HTAs targeting specific cellular enzymes —i.e., peptidylarginine deiminases (PADs)—endowed with a potent inhibitory activity against human beta-coronaviruses (HCoVs). Specifically, we show that infection of fetal lung fibroblasts and non-human kidney epithelial cells or human hepatoma-derived cells with HCoV-OC43 and SARS-CoV-2, respectively, leads to aberrant citrullination, a posttranslational modification associated with various inflammatory conditions, and upregulation of PAD4 isoform in human fetal lung fibroblasts. Most importantly, we show that targeting PADs, the cellular enzymes catalyzing protein citrullination, significantly reduces HCoV-OC43 and SARS-CoV-2 replication in vitro. Overall, our results demonstrate the potential efficacy of PAD inhibitors, namely Cl-amidine and its derivative BB-Cl-amidine, and GSK199, in suppressing HCoV infection, which may provide the rationale for the repurposing of this class of inhibitors for the treatment of COVID-19 patients.

230. Synthesis and Antiviral Evaluation of β-L-[5-(E-2-bromovinyl)-2-(hydroxymethyl)-1,3-(dioxolane-4-yl) uracil (L-BHDU) Prodrugs Against Varicella Zoster Virus (VZV)

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There is an urgent need for new antiviral agents with enhanced potency and specificity to treat herpes zoster caused by varicella-zoster virus (VZV) infection. We previously evaluated L-BHDU, β-L-1-[5-(E-2-bromovinyl)-2-(hydroxymethyl)-1,3-(dioxolan-4-yl)] uracil and demonstrated significant anti-VZV activity in three models of VZV replication: primary human foreskin fibroblasts (HFFs) and retinal pigment epithelial cells (ARPE-19), skin organ culture (SOC), and in SCID-Hu mice with skin xenografts. A prodrug approach was adopted to increase the potency, bioavailability and efficacy of L-BHDU against VZV. A series of amino esters, phosphoramidates, long-chain phospholipids, and phosphate ester prodrugs of L-BHDU were synthesized. Their potency was evaluated in ARPE-19 cells infected with VZV-ORF57-Luc, a wild type reporter virus. Amino ester prodrugs of L-BHDU were active. Phosphoramidates demonstrated moderate anti-VZV activities, while phosphate esters, POM-L-BHDU and POC-L-BHDU exhibited potent antiviral activity with EC_{50} 0.028 μM and EC_{50} 0.034 μM, respectively, without cellular toxicity (CC_{50} > 100 μM). Long-chain phospholipids octadecyloxyethyl-L-BHDU (ODE-L-BHDU) and hexadecyloxypropyl-L-BHDU (HDP-L-BHDU) also demonstrated significant activity with an EC_{50} range of 0.068 to 0.090 μM.

231V. Pre-clinical evaluation of LAVR-289, a novel potent CMV replication inhibitor

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Human cytomegalovirus (HCMV) infection is the leading cause of post-transplant complications and of infectious congenital neurological disability. Currently available treatments are highly toxic and resistances have been isolated. New effective therapies are needed especially to prevent and treat congenital infections. We investigated the mechanism of action and evaluated the efficacy of a novel acyclonucleoside phosphonate prodrug, LAVR-289. It blocks viral DNA replication measured from D0 to D5 by CMV/albumin multiplex PCR earlier than cidofovir, does not inhibit viral penetration or the production of the transactivator protein IE1 very early in the viral cycle. However, it does inhibit the production of the very early HCMV protein, IE-2 of 86 KDa, whose synthesis is regulated by certain late genes. LAVR-289 is effective against reference CMV strains with fibroblastic tropism AD169, or epithelial TB40/E and VHLE with an EC_{50} between 20 and 80 nM. In placental villus histoculture, the EC_{50} of LAVR-289 is 53 nM. The EC_{50} of cidofovir is 1uM on average. The cytotoxic concentration (CC_{50}) of LAVR-289 was tested on MRC-5 cells and ARPE-19 epithelial cells in combination with several molecules: aciclovir (EC_{50} CMV 40 uM), maribavir and letermovir. The CC_{50} is higher than 12 uM for growing cells (SI > 700) and is not reached for the combinations. LAVR-289 is an effective inhibitor of viral replication with low in vitro toxicity, and represent a new molecule for the treatment of congenital CMV infection.

Supported by the Région CVL (APR Finals), RTR FéRi2 and DGA/IAD (Denalpovir)

232. Antiviral Activity of Peptide A-3302-B Isolated From a Marine Bacterium Micromonospora sp. Against Herpes Simplex Virus Type 2.

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Herpes Simplex Virus type 2 (HSV-2) infections are a worldwide health concern, and there is still a great demand for new anti-herpetic agents primarily due to the emergence of resistance. In this context, active compounds from natural sources pose as interesting candidates for novel antiviral therapies. This work aimed to investigate the antiviral

activity of peptide A-3302-B, isolated from marine bacterium *Micromonospora sp.* strain MAG 9-7, against HSV-2 and other herpetic viruses. The chemical structure of the peptide was determined with ^{1}H and ^{13}C NMR spectra, as well as MS and MS/MS spectra. To investigate the antiviral profile and the mechanism of action of the peptide, we performed specific *in vitro* assays on cell culture lines. Results showed that peptide A-3302-B exerted a marked inhibitory activity against both a wildtype HSV-2 strain and an acyclovir-resistant HSV-2 strain, with similar EC_{50}s in the micromolar range. Mechanism of action studies demonstrated that the peptide did not inactivate extracellular viral particles, but it inhibited the steps of HSV-2 replicative cycle following the expression of late HSV proteins. In details, A-3302-B prevented the egress of HSV-2 virions from the infected cell and reduced cell-to-cell virus spread. Overall, we demonstrated for the first time the anti-HSV-2 activity of peptide A-3302-B isolated from a marine bacterium, identifying the peptide as an attractive candidate for the development of a microbicide for genital herpetic infections.

233. Optimization and Structure-Activity Relationship Studies of 1-Hydroxy-1,8-naphthyridin-2(1H)-one-based Derivatives as Potent Inhibitors for Hepatitis B Virus Replication

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Hepatitis B Virus (HBV) is a hepatotropic DNA virus that replicates by reverse transcription. HBV infects more than 200,000 people per year in the US, and worldwide greater than 250 million people are chronically infected. HBV is spread mainly through bodily fluids from an infected person to someone not infected. Current therapies primarily utilize nucleos(t)ide analog drugs that target the viral DNA polymerase and drive HBV below clinical detection limit but do not clear the infection. Reverse transcription needs the viral DNA polymerase to synthesize new DNA and the viral ribonuclease H (RNaseH). The viral RNaseH destroys the viral RNA pregenome after it has been copied into the DNA. Blocking RNaseH prevents synthesis of mature viral genomes and thus would prevent hepatitis B disease. To this effect, we are currently synthesizing, optimizing, and developing structure-activity relationships for a series of 1-hydroxy-1,8-naphthyridin-2(1H)-one-based small molecules in order to identify potential early drug discovery leads for treating chronic hepatitis B virus infections.

234. α-Hydroxytropolone HBV RNase H Inhibitors: Effects of Troponoids on Mitochondrial Function and Cytotoxicity

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The α-hydroxytropolones (αHT) are troponoid inhibitors of hepatitis B virus (HBV) replication that can target the HBV ribonuclease H (RNase H) with sub-micromolar efficacies. αHTs and related troponoids (tropones and tropolones) can be cytotoxic in cell lines as measured by MTS assays that assesses mitochondrial function. Earlier studies suggest that tropolones induce cytotoxicity through inhibition of mitochondrial respiration. Therefore, we screened 35 diverse troponoids for effects on mitochondrial function, mitochondrial:nuclear genome ratio, cytotoxicity, and reactive oxygen species (ROS) production. Troponoids as a class did not inhibit respiration or glycolysis, although the α-ketotropolone subclass did interfere with these processes. The troponoids had no impact on the mitochondrial DNA to nuclear DNA ratio after three days of compound exposure. Patterns of troponoid-induced cytotoxicity among three hepatic cell lines were similar for all compounds, but three potent HBV RNase H inhibitors were not cytotoxic in primary human hepatocytes. Tropolones and αHTs increased ROS production in cells at cytotoxic concentrations but had no effect at lower concentrations that efficiently inhibit HBV replication. Troponoid-mediated cytotoxicity was significantly decreased upon addition of the ROS scavenger N-acetylcysteine. These studies show that troponoids can increase ROS production at high concentrations within cell lines leading to cytotoxicity, but are not be cytotoxic in primary hepatocytes. Future development of αHTs as potential therapeutics against HBV may need to mitigate ROS production by altering compound design and/or by co-administration with ROS antagonists to ameliorate increased ROS levels.

235. Chimeric Capsid Inhibitors Carrying Proteolysis Targeting Moieties Induce HBV Capsid and Core Protein Degradation

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Hepatitis B virus (HBV) is an enveloped partially double stranded DNA virus that replicates via reverse transcription within the viral capsid. The HBV core protein is heavily involved in the viral replication cycle. Capsid assembly modulators (CAMs) can inhibit HBV replication by inducing the formation of aberrant non-icosahedral structures or morphologically intact empty viral capsids. First generation CAMs are highly effective against HBV and are in clinical trials. To date, ~20 resistance mutations have been identified that reduce the efficacy of these first-generation CAMs. Generating chimeric CAMs that employ additional mechanisms of inhibition will advance development of the next generation of CAMs. We covalently linked proteolysis targeting chimeras (PROTAC) carrying an E3 ubiquitin ligase ligand to the well-characterized CAM GLS4 to enhance the degradation of the viral core protein. We screened five groups of PROTAC-CAMs with different linkers and active or inactive E3 ubiquitin ligase ligands to determine if the active PROTAC molecule could elicit a reduction in both HBV capsid and core protein. Addition of a linker and an E3 ubiquitin ligase ligand to GLS4 increased the 50% effective concentration (EC_{50}) while maintaining low cytotoxicity. Treatment of HepDES19 cells replicating HBV with 2x EC_{50} of PROTAC-CAMs with an active E3 ubiquitin ligase ligand were capable of inhibiting core protein and capsid accumulation as measured by both western and native capsid blots. This proof-of-concept study showed that active PROTAC-CAMs possess an additional activity against HBV capsids and core protein.

236. Mechanism and Regulation of Intracellular Amplification of Hepatitis B Virus Covalently Closed Circular DNA and Implications in Antiviral Drug Development

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Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) exists as a minichromosome to transcribe viral RNAs and is refractory to the available antiviral medications. CCC DNA are synthesized from incoming viral genomic DNA during de novo infection and newly synthesized viral DNA in cytoplasmic progeny nucleocapsids. We demonstrated recently that *de novo* synthesis and intracellular amplification of cccDNA utilizes distinct cellular DNA polymerases to repair the relaxed circular viral DNA into cccDNA and is differentially regulated by cytoplasmic nucleases. Using a recombinant adenoviral vector expressing HBV or DHBV pgRNA to transduce cells, we showed that cccDNA intracellular amplification occurred in hepatocytes as well as multiple non-hepatic cell lines, which can be inhibited by viral DNA polymerase inhibitors or core protein allosteric modulators. While culturing cells in medium containing 2% DMSO significantly promoted viral DNA replication, but reduced cccDNA synthesis, treatment of cells with a small molecular compound B02 induced expression of heat shock proteins that significantly enhanced cccDNA intracellular amplification. Interestingly, DHBV cccDNA synthesized in human hepatoma cells was transcriptionally inactive. Knockdown of SMC5/6 complex that restricts HBV cccDNA transcription failed to activate DHBV cccDNA transcription, suggesting a distinct mechanism of episomal DNA silence. While the experimental systems established in this study provide robust assays for discovery and development of antiviral agents to eradicate or silence cccDNA, our results also imply that in addition to viral proteins, many host cellular factors regulate cccDNA synthesis and transcription and are thus potential therapeutic targets for the cure of chronic HBV infection.

237. The Design and Synthesis of Reverse Fleximers of L-Nucleoside Analogues

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Over 250 million people worldwide are affected by Hepatitis B virus (HBV) despite an existing, highly effective vaccine. Current HBV treatments include nucleos(t)ide analogues (NUCs), of which L-nucleosides have been proven

to be particularly effective. However, for chronic HBV (CHB), these treatments fail to fully eradicate the virus and must be continued lifelong to maintain viral suppression. One primarily hurdle of achieving a functional CHB cure is the development of drug resistance, an increasing risk with the current long-term NUC therapies. In 2001, Seley-Radtke introduced a novel class of modified nucleosides, "fleximers", which have proven to have both potent and broad-spectrum antiviral activity due to added flexibility afforded by splitting of the purine nucleobase via implementation of a single carbon-carbon bond between the imidazole and purine moieties. Later, this concept was expanded upon with "reverse fleximers" in which the pyrimidine moiety is bonded to the sugar instead. This reversal maintains the split purine system while also resembling substituted pyrimidines, allowing recognition by both purine and pyrimidine metabolizing enzymes. The added flexibility may also help overcome mutations within enzyme binding sites. This technology can be applied to current L-NUCs with proven potent anti-HBV activity as many of these compounds feature only a pyrimidine moiety, such as lamivudine, telbivudine, and clevudine. This combination of nucleoside modifications could thus result in compounds that possess not only potent anti-HBV activity but are also more resilient to drug resistance. The design and synthesis of such compounds are reported herein, along with computational docking studies.

238. Novel hepatoselective dihydroquinolizinones for HBV surface antigen (HBsAg) reduction

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One of the common aspects of chronic hepatitis B (CHB) is the high level of hepatitis B virus (HBV) surface antigen (HBsAg) in the bloodstream, and thus, the reduction of this antigenemia is considered one of the primary goals for CHB therapy. Although there are currently no approved methods to reduce HBsAg, a novel dihydroquinolizinone scaffold has been discovered to degrade HBV gene products, including HBsAg, in both tissue cultures and animal models. However, further development of Roche's lead compound RG-7834 (DHQ-1) has been ceased due to undisclosed toxicity concerns. Therefore, we have pursued the design and synthesis of hepatoselective DHQ derivatives, as compared to the systematic DHQ-1, in order to create a liver-targeting drug that has limited distribution to the blood and other body tissues.

239. Predicted Structure of the Hepatitis B Virus Polymerase Reveals an Ancient Conserved Protein Fold

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Hepatitis B virus (HBV) replicates by protein-primed reverse transcription. The virus kills chronically infects >250 million people and the dominant anti-HBV drugs are nucleos(t)ide analogs targeting the viral polymerase (P). P has four domains, the terminal protein (TP) that primes DNA synthesis, a spacer, the reverse transcriptase (RT), and the ribonuclease H (RNaseH). Despite being a major drug target and catalyzing a reverse transcription pathway very different from the retroviruses, P has resisted structural analysis for decades. Here, we exploited recent advances in protein structure prediction model the structure of P. The catalytic core formed a globular domain with a 3.75 Å RMSD relative to the HIV enzyme, had a nucleic acid binding groove spanning the two active sites, had all known active site motifs in positions analogous to homologous enzymes, and accommodated Mg^{++} ions in both active sites. Surprisingly, the TP domain wrapped around the catalytic core of P, with the priming residue poised over the RT active site. This model was validated using 30 years' of published mutational analyses and by docking RT and RNaseH inhibitors, RNA:DNA heteroduplexes, and the HBV epsilon RNA stem-loop that templates DNA priming. The HBV P fold, including the orientation of the TP, was shared with members of the *Hepadnaviridae* family from rodents to fish, and in P from a fish nackednavirus. Hepadnaviruses diverged from nakednaviruses >400 million years ago, indicating that the hepadnaviral P fold is ancient. These models will help guide drug development and structure-function studies into P's function.

240V. L-BHDU Prodrugs are Highly Effective Against Varicella Zoster Virus in vivo

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Herpes zoster, caused by varicella zoster virus (VZV), afflicts 1 in 3 people during their lifetime. There is a critical need for additional antivirals that are effective and safe. We evaluated L-BHDU (β-L-bromovinyl-hydroxymethyl-dioxolan-uracil) and its prodrugs against VZV in ARPE-19 cells, measuring EC_{50} values in the nanomolar range that were not cytotoxic. We previously showed that L-BHDU was effective against VZV in a humanized SCID mouse model when delivered continuously in Alzet pumps. However, it was unknown if the prodrugs were effective against VZV in vivo. Thus, we compared L-BHDU to its prodrugs, POM-L-BHDU, POC-L-BHDU, and octadecyloxyethyl-L-BHDU (ODE-L-BHDU), in the NuSkin mouse model. Adult human skin was obtained from reduction mammoplasties, and 1-cm^2 pieces were placed subcutaneously in athymic nude mice. After engraftment, xenografts were inoculated with VZV-ORF57-Luc and virus spread was measured by bioluminescence imaging. POM-L-BHDU and ODE-L-BHDU significantly reduced VZV spread when administered subcutaneously or orally, once daily, at 22.4 and 24.9 mg/kg respectively. L-BHDU and POC-L-BHDU did not prevent virus spread in vivo at equimolar doses on this treatment schedule. All compounds were well tolerated in mice with no signs of weight loss or distress. The effects of ODE-L-BHDU were durable after the treatment phase concluded. These results suggest that the POM-L-BHDU and ODE-L-BHDU modifications improve oral bioavailability and pharmacokinetics of the parent compound, and further PK/PD studies are currently underway. Future studies will address dose reductions and treatment schedules. These L-BHDU prodrugs are safe and effective, filling a demand for better antivirals against VZV.

246V. Differential Inhibition of PAPD5 and PAPD7 by Small-Molecule HBV RNA Destabilizers from Dihydroquinolizinone and Tetrahydropyrido[4,3-d]pyrimidine Chemical Series

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Noncanonical poly(A) polymerases PAPD5 and PAPD7 (PAPD5/7) stabilize HBV RNA through the viral post-transcriptional regulatory element (PRE). Inhibition of PAPD5/7 reduces HBV RNA, which in turn leads to suppression of viral replication and viral protein production, including HBsAg. In this study, representative PAPD5/7 inhibitors from a tetrahydropyrido[4,3-*d*]pyrimidine (THP-1) and a dihydroquinolizinone (DHQ, represented by AB-452) chemical series were evaluated against PAPD5/7 enzymes and HBV expressing cells. Biochemical data showed that THP-1 inhibited PAPD5/7 with similar efficiencies, while AB-452 was more active against PAPD5 when compared to PAPD7. THP-1, but not AB-452, inhibited PAPD4 enzymatic activities. Consistent with the biochemical results, AB-452 was more active against HBV-infected PAPD7-knockout cells when compared to wildtype and PAPD5-knockout cells, while THP-1 exhibited similar potencies across these cell lines. Furthermore, AB-452 inhibited HBsAg, HBeAg, HBV DNA, and HBV RNA with an EC_{50} that ranged from 1.4 to 4.6 nM in multiple HBV cell models. THP-1 reduced HBsAg and HBeAg with similar potencies but was ~10-fold less efficient against HBV RNA and HBV DNA in infected primary human hepatocytes. *In vitro* combination studies of AB-452 with capsid inhibitors or nucleoside analogs showed additivity to moderate synergy. Interestingly, analysis of intracellular HBV RNA revealed that pgRNA was more robustly degraded by AB-452 in the presence of capsid inhibitors, supporting potential combination strategies of HBV RNA destabilizers with capsid inhibitors.

260. Antiviral Activity of the Clinical Stage TLR7 Agonist Prodrug PRTX007 in a Murine Respiratory Syncytial Virus Animal Model

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PRTX007 is an oral prodrug of PRX034, a novel toll-like receptor 7 (TLR7) agonist that uniquely elicits interferon-mediated immune induction without inducing inflammation via the NF-kB pathway. Accordingly, pharmacologic response to PRTX007 in healthy humans for the first time supports the use of a TLR7 agonist in treatment of respiratory viral infections (RVIs). PRTX007's antiviral activity was assessed in standard cellular models of viral infection using conditioned media (CM) prepared by incubating human peripheral blood mononuclear cells with PRX034 and harvesting the supernatant. PRX034-derived CM effectively inhibits cytopathicity and viral replication in vitro against multiple RNA viruses, including respiratory syncytial virus (RSV). Most activity is mediated through pDC-derived interferons; potency is substantially greater than predicted by IFNα2 content alone. CM-pretreated cells are more protected than when CM is added with, or after addition of, virus. PRX034 alone has no effect in the absence of immune cells. CM plus presatovir (viral fusion inhibitor) exhibits additive benefit against RSV infection, approaching frank synergy where inhibition by either agent alone is modest. Tolerability and efficacy of PRX034 were evaluated in a murine model of RSV infection. PRX034 was delivered by IV administration of the prodrug PRX118, because PRTX007 conversion to PRX034 is inefficient in rodents. In this model, PRX034's antiviral activity was comparable with that achieved with intranasal IFNα or intranasal imiquimod, as assessed by viral RNA and viral titer from the lungs of infected animals. No adverse effects were observed. Further clinical investigation of PRTX007 for treatment of RVIs is warranted.

261. Unravelling the anti-influenza effect of flavonoids: Experimental validation of luteolin and its congeners as potent influenza endonuclease inhibitors

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The influenza virus causes severe respiratory infections across the human population and leads to nearly half a million deaths each year. A key protein within the viral infection cycle is the influenza polymerase – heterotrimeric enzyme with cap binding, polymerase, and endonuclease activities. The protein acidic (PA) subunit containing the endonuclease activity and is a target for anti-influenza therapies, such as the FDA-approved drug Xofluza. A general feature of endonuclease inhibitors is their ability to chelate Mn^{2+} and Mg^{2+} ions embedded in the enzyme's catalytic site. Flavonoids are natural compounds well known for their beneficial effects on health. Despite efforts to better understand their anti-influenza activity, there had not been clear consensus about their precise mode-of-action at cellular level. We have developed a high throughput screening assay based on AlphaScreen technology and validated a large set of flavonoids against PA N-terminal domain of pandemic isolate A/California/07/2009 H1N1. Submicromolar inhibitors were also evaluated by an *in vitro* gel-based endonuclease inhibitory assay, and these results corresponded to the data from the competitive AlphaScreen assay. The most potent inhibitors identified were luteolin with an IC_{50} of 72 ± 2 nM and its 8-C-glucoside orientin with an IC_{50} of 43 ± 2 nM. Using X-ray crystallography, we have analyzed four protein-ligand structures, including the I38T mutant variant, ranging from 1.9 Å to 2.5 Å resolution. Employing two distinct assays along with the structural work, we have identified and characterized the molecular mode-of-action of flavonoids in influenza-infected cells.

262V. Monitoring susceptibility of seasonal influenza A and B viruses to baloxavir in the United States, 2018-2021

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Baloxavir, a new influenza drug targeting the cap-dependent endonuclease activity of polymerase acidic (PA) protein, was approved in Japan and the U.S. in 2018, and more recently in other countries. CDC monitors the baloxavir susceptibility of influenza A and B viruses submitted to the national virological surveillance by analyzing PA sequences

for amino acid substitutions previously reported to reduce *in vitro* susceptibility by ≥3-fold. Additionally, sequence-flagged and representative viruses are tested phenotypically using HINT, a CDC-developed cell culture-based assay. Subtype/lineage-specific median EC_{50}s are determined and used to calculate the fold difference for each test virus. Notably, virological surveillance primarily focuses on transmission of drug-resistant viruses in communities and therefore viruses are typically collected before antiviral treatment. PA sequences of 5,918 viruses circulating during 2018-2021 influenza seasons were analyzed and I38L, E199G, L28P or I38V were detected in five influenza A viruses. Substitutions I38L and E199G conferred 4-8-fold elevation in EC_{50}s, whereas L28P did not affect drug susceptibility. Two viruses carrying I38V, a substitution previously associated with only 1-3-fold reduction, were not isolated. A rare substitution PA-K34R in the active site was detected in one virus, which displayed 4-fold reduced susceptibility. The remaining phenotypically tested viruses (n=552) were baloxavir susceptible (<3-fold). Our results differ from surveillance data in Japan, where 5% of influenza A viruses tested in 2018-2019 were baloxavir-resistant vs. 0.09% in the U.S. Although baloxavir-resistance among viruses circulating in the U.S. remains low, implementation of baloxavir testing at additional U.S. public health laboratories can improve pandemic preparedness.

263V. Characterization of influenza B viruses with reduced susceptibility to influenza neuraminidase inhibitors.

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We investigated the effect of 11 neuraminidase (NA) mutations identified through routine screening, on the susceptibility of influenza B viruses to the four registered neuraminidase inhibitors (NAIs) (oseltamivir, zanamivir, peramivir and laninamivir). Reverse genetics methodologies were used to introduce the 11 mutations into the NA of B/Brisbane/27/2016 (a B Victoria-lineage virus) or B/Yamanashi/166/98 (a B Yamagata-lineage virus). The effects of these mutations were analysed by an *in vitro* NAI assay. The T146K substitution in the NA of B Victoria and Yamagata-lineages resulted in a significant increase in the IC_{50} for peramivir (>1000-fold increase in the mean IC_{50} of sensitive viruses) with smaller increases for zanamivir and oseltamivir. A proline substitution (T146P) had a slightly lower (>700-fold) effect on the peramivir IC_{50} and also on the other NAIs. The presence of a second NA mutation at N169S combined with the T146P further increased the IC_{50} of peramivir (>7000-fold) and other NAIs. Synergistic effect was also confirmed for dual mutation G247D+I361V, which showed modest increase in the IC_{50} for oseltamivir (6-fold). Only one out of two RG-viruses with the NA mutation G108E could be rescued and it had a high IC_{50} against zanamivir (>4000-fold) and laninamivir (>7000-fold), but a lower IC_{50} against oseltamivir (>200-fold). NA mutations H101L, A200T, D432G, H439P and H439R were also confirmed to somewhat reduce the in vitro susceptibility of influenza B viruses to the NAIs. Overall, our study presented the potential impact of these mutations on the clinical performance of NAIs when used to treat influenza B infections.

265. Comparison of Four Enterovirus D68 Clinical Isolates from 2018 for Neurovirulence in Type I Interferon Receptor (IFNAR) Knock-out Mice

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Enterovirus D68 (EV-D68) was identified in 1962 as a causative agent of pediatric pneumonia. Since that time, EV-D68 has primarily been considered a respiratory virus with sporadic reports of neurological signs. During an outbreak of EV-D8 in 2014, cases of illness resembling acute flaccid myelitis (AFM) were temporally correlated with the increase in EV-D68 infections. Outbreaks of EV-D68 and increases in reported cases of AFM continue to occur in a biennially fashion. Over the decades since its discovery, EV-D68 has demonstrated the ability to evolve rapidly leading to increased transmissibility and neurovirulence. The goal of our research was to characterize four clinical isolates of EV-D68 obtained during the 2018 outbreak in the IFNAR mouse model. To compare the neurovirulence of these four isolates, we infected IFNAR mice with identical challenge doses of virus to evaluate mortality, neurological signs, and weight-loss after infection. Mortality was used as the primary indicator of neurovirulence for the four EV-D68 clinical isolates. The most virulent isolate, USA/2018-23201 (WA), showed 100% mortality following challenge with 1×10^6, 1×10^5, and 1×10^4 50% cell culture infectious doses ($CCID_{50}$) of virus. In mice infected with USA/2018-23216 (NY), with a 1×10^6 $CCID_{50}$ challenge dose, 75% mortality was observed with this Isolate, while only 40% and 20% mortality were observed for USA/2018-23201 (WA) and USA/2018-23263 (MN) isolates, respectively. These results demonstrate the neurovirulence of each EV-D68 isolate can be ranked as MD > NY > WA > MN.

[Supported by NIH contract HHSN272201700041I]

280V. Tissue replication and mucosal swab detection of Sosuga virus in Syrian hamsters in the absence of clinical disease

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Human infection with Sosuga virus (SOSV), a recently discovered pathogenic paramyxovirus, has been reported in one individual to date. In 2012, the patient developed clinical signs upon returning to the U.S. after 6 weeks of field work in South Sudan and Uganda. Severity of illness resulted in 2 weeks of hospitalization prior to recovery. SOSV was subsequently detected in bat tissues collected just prior to the onset of symptoms and in archived *Egyptian rousette* bat tissues collected at other locations in Uganda, indicating bats as a natural SOSV reservoir. To date, no animal models are under development for SOSV. Here, we describe initial characterization of experimental SOSV infection in Syrian hamsters by investigating the kinetics of virus in tissues over time and the corresponding clinical parameters and immunological responses. Five-week-old male and female hamsters were inoculated intranasally or intraperitoneally with recombinant wild-type virus based on the viral sequence obtained from the patient. Groups of animals were serially euthanized 1, 4, 8, 14 and 28 days post infection (dpi). Blood, tissues, and mucosal swabs were collected for clinical, virological, and immunological analyses at each timepoint. In addition, weight and temperature changes were assessed daily in animals euthanized at 14 and 28 dpi. Despite the absence of overt clinical signs, viral RNA was transiently detected in a variety of tissues and swab samples, indicating subclinical infection. These data provide a basis for further efforts to develop infection and disease models of SOSV for pathogenesis studies and medical countermeasure development efforts.

281. Synthesis and evaluation of Hydroxymethyl-propyl-phosphonates-diphosphates (HPMPG/T)

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Viral infections and diseases, a continuous and currently highly relevant issue that has determined our daily lives for almost 2 years in the case of the SARS-CoV-2 pandemic. Therefore, developing antiviral strategies, including discovery of new and improved antiviral compounds is a top priority target.

A widely applied strategy is the use of nucleoside or nucleotide analogs. Herein, acyclic nucleotide phosphonates (ANP), developed by the research group of Antonín Holý in the 1980s, opened up a new field. These compounds, with a stable phosphorus-carbon-bond, mimic nucleoside monophosphates and are stable against chemical or enzymatic dephosphorylation. Nonetheless, these compounds need to be phosphorylated intracellulary to the corresponding triphosphate analog, the active metabolite, which is often a hurdle. HPMPC (Cidovofir) is a well-known ANP with broad *in vitro* antiviral activity. Nonetheless, similar compounds with a different nucleobase, such as HPMPT or HPMPG, don't show high antiviral activity even in a prodrug form. This leads to the question whether these compounds are not enzymatically phosphorylated or are a poor substrate for HIV reverse transcriptase. Our lab has extensive knowledge in the field of nucleotide synthesis and our Tri*PPP*ro-technology successfully delivers nucleoside triphosphates into cells. Here, we transferred this knowledge to HPMPs including nucleobases G and T. HPMPG/T-diphosphates enable studies regarding their substrate properties towards HIV-RT. Their prodrugs are able to overcome metabolic bottlenecks and hence achieve favorable antiviral activities.

282V. Optimization of a Miniaturized, Cell-based, Screening Assay to Discover Rabies Virus Antivirals

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Rabies virus (RABV) causes a fatal neurological disease, mainly in children. Although Post Exposure Prophylaxis (PEP), in the form of neutralization rabies immunoglobulins (RIG) and vaccination, results in efficient protection, the financial cost of RIG and their limited availability in remote areas remains a challenge. A selective antiviral that helps to prevent rabies disease progression, or that is effective in the treatment of clinical rabies would therefore be a game changer. We developed a high-throughput assay on a high-content imaging platform and used this to screen a library of repurposing drugs. The RABV infection and cell viability measurements in BHK cells were optimized using the fluorescence of mCherry-RABV and the fluorescence of Hoechst staining of the cell nuclei. The average Z' factor and coefficient of variation (CV) obtained after a 4-day RABV exposure were 0.96 and 3.6%, respectively. A repurposing drug library (~3200 compounds) was screened and 10 hits were selected (less than 30% RABV infection and more than 50% cell viability). These were confirmed in a 96-well plate protocol, which was also optimized for antiviral screening with a Z' factor of 0.95 and a CV of 1.9%. In this way, we confirmed 1 compound, Salinomycin, as an endocytosis inhibitor of RABV. The hit rate of this screening campaign was thus 0.3%, and the confirmation rate was 10%. In conclusion, we developed and optimized a miniaturized, cell-based, large-scale antiviral screening assay for RABV. This can provide starting points for the discovery of new RABV antivirals.

283V. Discovery and Mechanistic Investigations of Phenylalanine Derivatives as Novel HIV-1 Capsid Inhibitors

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HIV-1 capsid represents an appealing antiviral target[1]. PF74, a phenylalanine derivative, binds to the NTD-CTD interface of capsid hexamer with unique mechanism[2]. To improve its antiviral activity and metabolic stability, the structure-based drug design and the parallel derivatization reaction were employed to quickly construct the privileged fragment-derived compound library. Then, antiviral activity screening, mechanistic investigations and metabolic stability assessments culminated in the discovery of novel capsid inhibitors (Figure 1). Notably, 11L with a piperazinone moiety exhibited anti-HIV-1 activity about 5-fold better than PF74[3]. Q-c4 was designed as a dimerized phenylalanine derivative, aiming to occupy the larger area in the NTD-CTD interface[4]. 7u with a benzothiazole moiety displayed greater metabolic stability in human liver microsomes with half-life ($t_{1/2}$) 109-fold that of PF74[5]. Moreover, surface plasmon resonance studies and molecular dynamics simulations on representative compounds confirmed the drug target and the binding site. The single-round infection assays demonstrated that these inhibitors exhibited antiviral activities with a dual-stage inhibition profile. These studies provide insights and serve as a starting point for subsequent medicinal chemistry efforts in optimizing these promising HIV-1 inhibitors.

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287. Activity of Islatravir Against Diverse Primary Isolates of HIV-1 and Putative Resistant and Hypersensitive Mutants

Maria E. Cilento, M.S.[1], Philip R. Tedbury, Ph.D. [1], Obiaara B. Ukah, Ph.D.[2], Eiichi N. Kodama, Ph.D.[3], Hiroaki Mitsuya, Ph.D.[4], Michael A. Parniak, Ph.D.[5], Karen A. Kirby, Ph.D. [1], Stefan G. Sarafianos, Ph.D. [1]

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Islatravir (ISL) is a novel NRTI that retains a 3′-OH, is efficiently activated by kinases and binds reverse transcriptase (RT) with an affinity comparable to the native nucleotide. It inhibits RT translocation after ISL incorporation in the nascent viral DNA chain. It is important to characterize the activity of ISL against viruses carrying resistance mutations that emerge during tenofovir-based (K65R) or 3TC/FTC-based (M184V) therapies. In HIV-1 subtype B, M184V imparts low resistance to ISL, whereas K65R causes hypersensitivity to this antiviral. Limited data are available on the potency of ISL against non-subtype B HIV. Here we determined EC50s for HIV subtypes A, B, C, and circulating recombinant forms (CRF) 01_AE and 02_AG. We found that K65R imparted hypersensitivity to ISL in all isolates whereas M184V showed only minimal resistance to this antiviral. Interestingly, M184V increased ISL resistance (less than 20-fold) in some non-B subtypes relative to subtype B. Surprisingly, whereas K65R suppressed the M184V-based ISL resistance in most subtypes, it enhanced ISL resistance in HIV-1AEK65R/M184V compared to HIV-1-AEM184V. Upon further investigation, we found HIV-1-AE contains S68G, a mutation that emerges during TDF/TAF-treatment. Notably, the most common G68S substitution restores the resistance profile of HIV-1-AEK65R/M184V similar to HIV-1-AEM184V. Hence, ISL is a promising drug for the treatment of patients infected with all subtypes of HIV-1, including those who have failed existing tenofovir- and 3TC/FTC-based therapies. Additionally, ISL may be suitable for combination with tenofovir, owing to the mutually exclusive resistance profiles.

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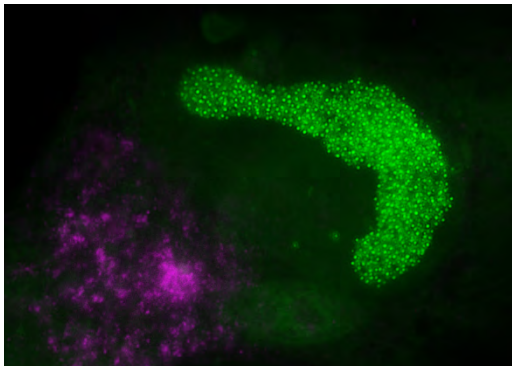
NeoVirTech SAS is a biotechnology company incorporated early 2014 that develops autofluorescent viruses and viral vectors for imaging and discovery of novel antiviral drug candidates in the fields of human health, animal health and biodefense markets. NeoVirTech successfully closed multiyear programs backed by biotech and large pharma companies, TTOs and government agencies. The company provides the most diverse catalog of viruses with a One Health vision incorporating its proprietary ANCHOR™ technology, the only non-invasive technique to detect viral DNA replication inside living cells using imaging approaches. Having the ability to visualize virus infection and replication allows fast and efficient discovery of antiviral molecules and investigation of mechanism of action. Since the Covid-19 pandemic, the company is actively involved in SARS-CoV-2 screening campaign (pre-VOC, Delta and Omicron) and measurement of disinfection procedures. NeoVirTech works in BSL2, BSL2+ and BSL3 environment combined with A2 and A3 animal facilities for *in vivo* efficacy studies. Want to challenge your molecule on our virus collection ? contact us at: contact@neovirtech.com, www.neovirtech.com



Primary human cell infected with hCMV ANCHOR™ (green). Each fluorescent spot corresponds to a copy of the viral DNA. Cell is counterstained for gB (magenta).

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