PROGRAM & ABSTRACTS

ICAR2024

37TH International Conference on Antiviral Research (ICAR)

HOSTED BY **International Society for Antiviral Research (ISAR)**

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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 37th 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 May 10, 2024

ICAR2024

PREMIER PLATINUM

GILEAD
Creating Possible

PLATINUM

Pfizer

VYRAMED

DIAMOND

ATEA Pharmaceuticals

pandemic antiviral discovery

sanofi

SAPPHIRE

assemblybio

GOLD

The Stephan Urban Foundation

SILVER

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European Virus Archive

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Institute for Glycomics, Griffith University

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RFS Family Foundation

BRONZE

Arbutus BioPharma

Baruch S. Blumberg Institute

Drugs for Neglected Diseases initiative

FUJIFILM Toyama Chemical CO., LTD

IOCB TECH s.r.o.

NeoVirTech, SAS

Oxeltis

Virtual Platform Information

ICAR2024

ICAR2024 will offer both in-person and virtual programming options. Please find below details to assist you with navigating the ICAR2024 virtual platform.

>> How do I access the virtual platform?

All registered attendees (onsite and virtual) will receive log-in details on Monday, May 20. The ICAR2024 virtual platform will be available as a mobile event app or attendee website accessible from your computer.

>> When will conference content be available?

Content will be available to all registered attendees starting Monday, May 20, 2024.

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

All registered attendees will be able to access on-demand content through June 24, 2024.

>> Will the oral sessions be streamed live?

The onsite sessions will not be streamed live on the virtual platform. Virtual attendees will be able to view recordings of the onsite oral sessions.

>> Will the live sessions be recorded?

If you are unable to attend in-person, the live onsite sessions will be recorded and posted for on-demand viewing within 24-48 hours following the live in-person session. Please note that the Women in Science Roundtable and Career Development Interactive Workshop will 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. There will be no live Q&A for virtual posters. Click on the "Posters" tab, to view a list of all the posters.

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

For each poster, you will be able to connect with the Poster Presenter by clicking on their name at the bottom of the page to send them a mesage. Presenters are expected to monitor their posters and chat messages at least twice daily during the conference week. We encourage attendees to leave feedback, questions, or ask to be contacted for more information, so that they know you visited. There will be no live Q&A for virtual posters.

>> How do I learn more about the virtual platform?

Please visit our website and click on the Frequently Asked Questions page. This page will be updated frequently with details and tips for ICAR2024 to help you become familiar with the virtual platform.

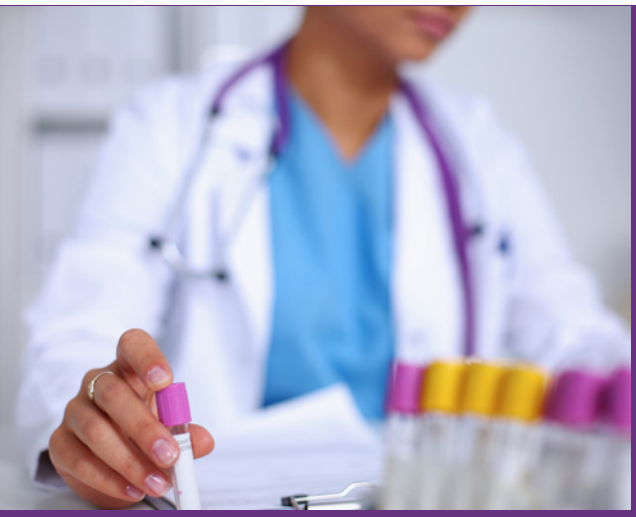
Pre-Conference Symposium

Sunday, May 19 | 1:00 – 6:00 PM AEST at GRIFFITH UNIVERSITY GOLD COAST CAMPUS

An Australasian Virology Mini Symposium – *Advancing Knowledge, Protecting People*

- **Prof Rowena Bull**
  University of New South Wales and current President of the Australasian Virology Society
- **Prof Linfa Wang**
  Duke-NUS Medical School
- **Prof Paul Young**
  University of Queensland

Free-of-charge to ICAR2024 registrants.
Separate registration is required to attend the symposium.



SUPPORTED BY Pfizer

Women in Science Roundtable Discussion

MONDAY, MAY 20 | 12:00 – 1:45 PM AEST | PHOENIX ROOM

The WIS Committee is excited to announce the 12th Annual Women in Science Roundtable. This session, the first event on the first day of ICAR, will be held on Monday, May 20, from 12:00–1:45 PM. Check-in will occur from 12:00-12:30 PM. It is open to both women and men, and will feature discussions on the challenges and opportunities encountered by women scientists while navigating the twists and turns of career progression in today's environment. Food and beverages will be provided. Come join your antiviral colleagues for a fun and educational experience!

This event is free and open to everyone, but attendance is limited to 80 participants.

This event is at capacity. If you have joined the waitlist, you will be notified if there are any openings.

Opening Session and Plenary Speakers

MONDAY, MAY 20 | 2:00 – 4:15 PM AEST | NORFOLK BALLROOM

- **Prof. Edward Holmes, FAA, FRS**
  The University of Sydney, Australia
  Virus Emergence at the Human-Animal Interface
- **Dr. Jenny Low, MBBS, MPH**
  Singapore General Hospital, Duke NUS Medical School
  Bringing Antivirals to the Clinic: Challenges and Opportunities

Opening Reception

MONDAY, MAY 20 | 5:30 – 6:30 PM AEST | RELISH GRILL AND BAR

Following the opening session, all onsite attendees are invited to join us at the Opening Reception to mix and mingle with friends and colleagues to kick-off **ICAR2024**!

PeckaKucha Competition

TUESDAY, MAY 21 | 10:00 – 11:00 AM AEST | NORFOLK 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.

Career Development Interactive Workshop

TUESDAY, MAY 21 | 12:30 – 1:30 PM AEST | PHOENIX ROOM

The ICAR career development session will be an interactive Career Roundtable this year. The Career Roundtable will give the opportunity to registered attendees to meet established researchers who will provide their unique perspectives on career development, professional pitfalls, and scientific opportunities for trainee scientists. The experienced researchers were chosen to reflect a myriad of career paths and experiences (academia, industry, government, NGO, etc.). It is also an opportunity for early career researchers to meet their peers.Tables will be organized by different career paths and will enable attendees to interact with several senior scientists during short sessions in a comfortable small group setting. The Career Roundtable will be followed by a short, informal networking moment. ***Pre-registration is required.***

This event is at capacity. If you have joined the waitlist, you will be notified if there are any openings.

Late-breaking Oral Presentations

Wednesday, May 22
9:10 – 10:00 AM AEST
PHOENIX ROOM

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

Closing Event

Thursday, May 23
7:00 – 10:00 PM AEST
NORFOLK BALLROOM AND POOLSIDE

Gather poolside to celebrate another ICAR Aussie style with an Australian BBQ. The winners of the Poster Awards and PechaKucha Competition will be announced and the TCFF Awardees will also be recognized.

Schedule-at-a-Glance

**All times are listed in Australian Eastern Standard Time.*

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The in-person sessions in Gold Coast will be recorded and available via the virtual platform 24-48 hours after the live session. All posters (even those 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.

Sunday, May 19, 2024

TIME *(AEST)*	EVENT	LOCATION
1:00 PM – 6:00 PM	**Pre-Conference Symposium: An Australasian Virology Mini Symposium – Advancing Knowledge, Protecting People** *Separate registration required.*	GRIFFITH UNIVERSITY GOLD COAST CAMPUS

Monday, May 20, 2024

TIME *(AEST)*	EVENT	LOCATION
12:00 PM – 1:45 PM	**Special Event: Women in Science Roundtable***	PHOENIX
2:00 PM – 4:15 PM	**Opening Session and Plenary Session**	NORFOLK BALLROOM
4:15 PM – 4:30 PM	**Break**	NORFOLK FOYER
4:30 PM – 5:30 PM	**Gertrude Elion Memorial Award Lecture**	NORFOLK BALLROOM
5:30 PM – 6:30 PM	**Opening Reception***	RELISH GRILL AND BAR

**Session/Event will not be recorded. Available to in-person attendees only*

Tuesday, May 21, 2024

TIME *(AEST)*	EVENT	LOCATION
8:30 AM – 9:15 AM	**William Prusoff Memorial Award Lecture**	NORFOLK BALLROOM
9:15 AM – 10:00 AM	**Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness**	NORFOLK BALLROOM
10:00 AM – 11:00 AM	**PechaKucha Competition**	NORFOLK BALLROOM
11:00 AM – 11:15 AM	**Break**	NORFOLK FOYER
11:15 AM – 12:15 PM	**Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness** *(continued)*	NORFOLK BALLROOM
12:15 PM – 1:45 PM	**Lunch** *(included for all conference registrants)*	RELISH GRILL AND BAR
12:30 PM – 1:30 PM	**Special Event: Career Development Roundtable**	PHOENIX
1:45 PM – 2:30 PM	**Diversity in Science and Excellence Award Lecture**	NORFOLK BALLROOM
2:30 PM – 3:30 PM	**Coronaviruses, Influenza, RSV, and Other Respiratory Viruses**	NORFOLK BALLROOM
3:30 PM – 3:45 PM	**Break**	NORFOLK FOYER
3:45 PM – 5:00 PM	**Coronaviruses, Influenza, RSV, and Other Respiratory Viruses** *(continued)*	NORFOLK BALLROOM
5:00 PM – 7:00 PM	**Poster Session 1*** *(Light food and beverages provided)*	MONACO AND SIFU

Wednesday, May 22, 2024

TIME *(AEST)*	EVENT	LOCATION
8:30 AM – 9:10 AM	**Chronic, Latent, and Persistent Viruses - Retroviruses and Herpesviruses**	NORFOLK BALLROOM
9:10 AM – 10:30 AM	**Late-breaking Oral Presentations and Hepatotropic and GI Viruses**	NORFOLK BALLROOM
10:30 AM – 10:45 AM	**Break**	NORFOLK FOYER
10:45 AM – 12:15 PM	**Arboviruses**	NORFOLK BALLROOM
12:15 PM – 2:15 PM	**Poster Session 2*** *(Lunch provided)*	MONACO AND SIFU

**Session/Event will not be recorded. Available to in-person attendees only*

Thursday, May 23, 2024

TIME *(AEST)*	EVENT	LOCATION
8:30 AM – 9:20 AM	**Machine Learning and Computational Approaches for Antiviral Research**	NORFOLK BALLROOM
9:20 AM – 10:20 AM	**Coronaviruses, Influenza, RSV, and Other Respiratory Viruses**	NORFOLK BALLROOM
10:20 AM – 10:35 AM	**Break**	NORFOLK FOYER
10:35 AM – 11:25 AM	**Coronaviruses, Influenza, RSV, and Other Respiratory** *(continued)*	NORFOLK BALLROOM
11:25 AM – 12:00 PM	**Chronic, Latent, and Persistent Viruses – Retroviruses and Herpesviruses**	NORFOLK BALLROOM
12:00 PM – 12:15 PM	**ISAR Annual Business Meeting**	NORFOLK BALLROOM
12:15 PM – 1:45 PM	**Lunch** *(included for all conference registrants)*	RELISH GRILL AND BAR
1:45 PM – 2:30 PM	**Women in Science and Excellence Award Lecture**	NORFOLK BALLROOM
2:30 PM – 3:30 PM	**Arboviruses**	NORFOLK BALLROOM
3:30 PM – 3:45 PM	**Break**	NORFOLK FOYER
3:45 PM – 4:45 PM	**Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness**	NORFOLK BALLROOM
4:45 PM – 5:00 PM	**Break**	NORFOLK FOYER
5:00 PM – 6:00 PM	**Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness** *(continued)*	NORFOLK BALLROOM
7:00 PM – 10:00 PM	**Closing Event***	NORFOLK BALLROOM & POOLSIDE

Friday, May 24, 2024

TIME *(AEST)*	EVENT	LOCATION
9:00 AM – 10:20 AM	**Hepatotropic and GI Viruses**	NORFOLK BALLROOM
10:20 AM – 10:45 AM	**Break**	NORFOLK FOYER
10:45 AM – 11:15 AM	**Shotgun Presentations**	NORFOLK BALLROOM
11:15 AM – 12:30 PM	**Chronic, Latent, and Persistent Viruses – Retroviruses and Herpesviruses**	NORFOLK BALLROOM

**Session/Event will not be recorded. Available to in-person attendees only*

GERTRUDE ELION MEMORIAL AWARDEE

Johan Neyts, PhD

My Battle Against Viruses

Johan Neyts is full professor of Virology at the **University of Leuven (KU Leuven), Belgium**. He teaches virology at the medical school and at the school of dentistry. His lab has a long-standing expertise in the development of antivirals strategies and drugs against emerging and neglected viral infections such as dengue and other flaviviruses, Chikungunya and other alphaviruses, enteroviruses, noroviruses, HEV and rabies and is as well intensively involved in the development of antiviral strategies against SARS-CoV2. An ultrapotent pan-serotype dengue inhibitor developed in his laboratory and at the Centre for Drug Design & Development (www.cd3.be) is currently in clinical development at Janssen Pharmaceutica (J&J). A second focus is on the development of the PLLAV (Plasmid Launched Live Attenuated Virus) vaccine technology, which is based on the yellow fever virus vaccine as a vector. It allows to rapidly engineer highly thermostable vaccines against multiple viral pathogens. Johan is a past-president of the International Society for Antiviral Research. He is the co-founder of KU Leuven spin-off companies AstriVax www.astrivax.com and Okapi Sciences. He is responsible for the Belgian VirusBank platform www.virusbankplatform.be , an investment of the Belgian Federal Government in epidemic/pandemic preparedness. He published >640 papers in peer reviewed journals (WoS H-index WoS 79, Google Scholar H-index 101, dd October 2023) and received multiple national and international awards. He has given ~330 invited lectures and hundreds of interviews to lay-press.

WILLIAM PRUSOFF MEMORIAL AWARDEE

Jessica Spengler, DVM, PhD, MPH

Viral Hemorrhagic Fevers: Challenges and Gains of Animal Model Research for Pre-Clinical Vaccine and Antiviral Screening

Dr. Jessica Spengler received her MPH. in infectious diseases in 2004 from the University of California, Berkeley, and then completed a California Epidemiologic Investigation Service (Cal-EIS) fellowship with the Vector-Borne Disease Section of the California Department of Health from 2004–2005. She received her PhD (2011) and DVM. (2012) from the University of California, Davis. Her graduate research on innate immune evasion by hantaviruses was performed on-site with Dr. Heinz Feldmann in the Special Pathogens Program at the Public Health Agency of Canada (Winnipeg, Manitoba), and at the NIH Laboratory of Virology, Rocky Mountain Laboratories (Hamilton, Montana). Since 2012, Dr. Spengler has worked with the Viral Special Pathogens Branch at the **Centers for Disease Control and Prevention** in Atlanta, Georgia. Currently, she directs a translational research program utilizing biosafety level 3 and 4 laboratory facilities to identify, prevent, ameliorate, and control high-hazard zoonotic viral pathogens, including Ebola, Marburg, Nipah, Crimean-Congo hemorrhagic fever, and Lassa fever viruses. This program investigates viral pathogenesis, develops animal models of disease, and conducts in vivo screens of therapeutic and vaccine candidates for high-containment, high-consequence viral pathogens. Dr. Spengler is presently an Editor for Antiviral Research and has been actively involved in ISAR activities since 2016, serving on the Women in Science Committee, assisting in communications and outreach, and as a member of the ISAR Board of Directors.

WOMEN IN SCIENCE AND EXCELLENCE AWARDEE

Judith Breuer, MD, FRCPath, FMedSci

From Target to Treatment

Judith Breuer is Professor of Virology at **UCL** and Clinical lead for Virology at **Great Ormond Street Hospital for Children**. Her research interests centre on precision medicine approaches to improved treatment of infectious diseases. These include pioneering high throughput pathogen sequencing directly from clinical material for identification of pathogen genetic determinants of clinical disease and understanding the actions of mutagenic drugs. Professor Breuer has exploited the pathogen genomic data to describe the landscape of genetic variation particularly for herpesviruses (VZV, CMV and EBV) and norovirus, and to develop evolutionary models that better elucidate the role of pathogen variation in transmission and disease. She has also worked for many years on VZV and the live vOka vaccine, generating insights into VZV natural history and pathogenesis. Her work led to the discovery of the VZV latency transcript (VLT). More recently she has uncovered the potential basis for vOka attenuation and in the process identified potential targets for broad spectrum antivirals against viral skin infections. Prof Breuer has developed diagnostic metagenomic methods for pathogen detection and discovery in patients with undetected infections of brain and other tissues. This has led to changes in the management of encephalitis particularly in the immunocompromised and the establishment of a pipeline to evaluate repurposed drugs against RNA viral infections. She recently used metagenomics to identify adeno-associated virus 2 as the cause of the unexplained hepatitis that occurred worldwide in children in 2022. This work has now been extended to investigating hepatitis associated with AAV gene therapy and the putative role played by co-infection with herpesviruses. Professor Breuer is a Fellow of the Academy of Medical Sciences, a member of the UK JCVI committees on VZV and HPV vaccines and the UK Polio eradication committee. She chairs the UKHSA Definitions of Immunosuppression Group.

DIVERSITY IN SCIENCE AND EXCELLENCE AWARDEE

Nancie Archin, PhD

Chasing an HIV Cure: The Intersection of Biological Sex and Latency Reversal

Dr. Nancie Archin is an Assistant Professor in the Infectious Diseases Division/ Department of Medicine at the **University of North Carolina at Chapel Hill**. She received her BS degree from Stony Brook University in New York and her PhD from the Department of Microbiology and Immunology at the University of Texas Health Sciences Center at San Antonio, Texas where she studied HSV-1-induced acute retinal necrosis syndrome under the supervision of her graduate advisor, Dr. Sally Atherton. She then joined the laboratory of Dr. David Margolis at UT Southwestern where she began her studies of HIV latency. Since joining the faculty at UNC, she has worked to move laboratory observations into clinical testing and applications. She has a strong and extensive publication track record on persistent HIV research, including the first study to show that HIV latency can be disrupted in vivo , studies of the reservoir in T cell subsets, studies to delineate the basic mechanisms of HIV latency, and other translational studies on using novel molecules combined with immunotherapy for latency clearance. Her laboratory's current research focus includes using molecular biology and biochemical methods to 1) define sex-specific and other factors that contribute to HIV persistence in people living with HIV with a particular focus on women, 2) define modalities to disrupt latency and clear latently infected cells, and 3) apply these observations in clinical settings.

The 2024 Chu Family Foundation Scholarship Awardees

ICAR2024

ISAR and The Chu Family Foundation (TCFF) Committee are pleased to announce the winners of the 2024 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.

2024 TCFF AWARDEES

Sofía Maldonado

UNIVERSITY OF BUENOS AIRES, ARGENTINA

Biochem Sofia Maldonado, a passionate PhD student from the University of Buenos Aires (UBA) Argentina, is dedicated to fighting viruses with global health impact. Her research centers on developing new antiviral treatments using nano-micelle drug formulations. This innovative approach targets viruses like Dengue, Yellow Fever, and Zika, all posing significant health concerns worldwide. Sofia's promising formulation has shown effectiveness in inhibiting the replication of these viruses, but to further refine it, she needs to unlock its mechanism of action.

Funded by The Chu Family Foundation Scholarship, Sofia is embarking on a crucial research 6-months stay at the Laboratory "Architecture et Fonction des Macromolécules Biologiques-AFMB" in Marseille, France. Under the guidance of Dr. Karine Alvarez, she will delve into the intricacies of the drug's antiviral properties. Her primary objective is to investigate how the formulation interacts with the viruses' proteins, essentially understanding how it disrupts their replication cycle.

Through a series of experiments, Sofia aims to pinpoint the drug's interaction with the viral RNA polymerase, an enzyme critical for viral reproduction. By evaluating its ability to decrease the enzyme's activity, she hopes to establish a clear picture of the drug's antiviral mechanism.

The collaborative efforts between her university and the AFMB Laboratory lay the groundwork for a lasting partnership, paving the way for future research initiatives that address global health challenges. This international exchange embodies the spirit of scientific progress, where knowledge and innovation combine to combat infectious diseases and improve global health outcomes.

Agostina Belén Marquez

UNIVERSITY OF BUENOS AIRES – CONICET, ARGENTINA

Agostina Marquez is a biochemist graduated from the Universidad Nacional de Mar del Plata, Argentina. In 2020, she was awarded a scholarship to pursue her doctoral studies at the University of Buenos Aires, Argentina. During the first years of her PhD studies, due to experiments carried out in silico and in vitro, their research group determined that cannabidiol (CBD), the principal non-psychoactive cannabinoid found in Cannabis sativa plants, has broad-spectrum antiviral activity. Currently in her fourth year of the doctoral program, Agostina's research focuses on elucidating the mechanism of action of CBD during flavivirus infections. The TCFF scholarship will enable her to undertake an internship at Monash University in Melbourne, Australia. The internship aims to investigate the impact of CBD treatments on the nucleocytoplasmic transport of the dengue virus capsid protein. Outside of her research, Agostina enjoys traveling, immersing herself in new cultures, and swimming.

Special Thank You to Our Local Hosts

Lara Herrero
Griffith University

Subhash Vasudevan
Duke-NUS Medical School

Mark von Itzstein
Griffith University

Sonja Best, PhD

Beyond Retroviruses: Restriction of Flavivirus Replication by TRIM5α

Sonja Best, PhD is currently the Chief of the Laboratory of Neurological Infections and Immunity (LNII) at the Rocky Mountain Laboratories (RML) campus of the **National Institute of Allergy and Infectious Diseases (NIAID, NIH)**. Her research group, called the Innate Immunity and Pathogenesis Section, focuses on understanding the host-pathogen interface associated with the intrinsic antiviral response of cells to emerging RNA viruses. Dr. Best earned her PhD from Australian National University in Australia where she examined pathogenesis of myxoma virus. She then joined the RML, NIAID, where she conducted postdoctoral research focusing on the role of host innate immunity in viral pathogenesis prior to establishing her independent laboratory. Dr. Best received tenure and was promoted to Senior Investigator in 2017. She received a Presidential Early Career Award for Scientists and Engineers (PECASE) and currently serves on multiple Editorial boards including Journal of Virology, PLoS Pathogens, and Science Translational Medicine.

Larissa Dirr, PhD

Drug Discovery Efforts Towards Human Metapneumovirus

Dr. Larissa Dirr is a current NHMRC Peter Doherty Fellow and an Early Career Research Leader at the **Institute for Glycomics, Griffith University**. She completed her Master thesis at the University of California San Diego, USA in Natural Product Chemistry and her Doctor of Philosophy degree in Virology and Structural Biology at Griffith University, Australia under the guidance of Prof. Mark von Itzstein. She then has worked as the lead Structural Virologist towards the development of a new class of parainfluenza drug candidates, where she is a co-inventor of three international patents. The parainfluenza technology has been licensed to Grand Medical Pty to deliver in a major co-development program the first human parainfluenza virus drug to market. More recently, her independent research focuses on understanding the role of viral glycoproteins involved in infection and spread to define antiviral targets and design new antiviral inhibitors for a range of respiratory viruses, including SARS-CoV-2, influenza virus and human metapneumovirus. She also uses a multi disciplinary approach to understand how viruses interact with host glycan receptors located at the cell surface. She is a leading member of the German Australian iCAIR (Fraunhofer International Consortium for Anti-infective Research) project that works towards new anti-infective therapies. She has been awarded the Queensland Protein Group Ross Smith Early Career Research Medal in 2022 and promotes women in science through the Glycomics Circle and the Qld STEM Education Network.

Jennifer E. Golden, PhD

Medicinal Chemistry Optimization and Therapeutic Efficacy of 2-Pyrrolidinoquinazolinones in Lethal Murine Models of Venezuelan and Eastern Equine Encephalitis Viruses

Dr. Jennifer E. Golden is an Associate Professor in the Division of Pharmaceutical Sciences and the Department of Chemistry at the **University of Wisconsin-Madison**. She also serves as the Associate Director of the UW-Madison Medicinal Chemistry Center and the Chair of the Early Career Board of ACS Medicinal Chemistry Letters. Trained as a synthetic medicinal chemist, Jennifer completed her PhD in medicinal chemistry at the University of Kansas (Prof. J. Aube, 2002) and postdoctoral work at Stanford University (Prof. P. Wender, 2004) before joining Amgen as a small molecule drug discovery scientist working in inflammatory and neurological disease. She was recruited to help establish an NIH-sponsored, US-wide academic drug discovery network at the University of Kansas where she served as the Assistant Director of the KU Specialized Chemistry Center. There, she directed the development of novel chemical probes across a diverse project portfolio before establishing her independent research group at Wisconsin in 2015. As such, Dr. Golden brings nearly 20 years of synthetic medicinal chemistry experience from a career spanning industry and academia. At UW-Madison, she has pioneered new synthetic chemistry methods aimed at efficiently building unique drug-like, heterocyclic architecture that is screened and advanced in medicinal chemistry campaigns. Further, she has established robust, well-funded, multidisciplinary drug discovery teams and a research platform that integrates the design, synthesis, and optimization of novel anti-infective agents aimed at improving human health scenarios for which limited, or no therapeutic options exist. Her highly collaborative programs have been directed at encephalitic alphavirus intervention (e.g. VEEV, EEEV) and parasitic diseases (e.g. PAM infection from N. fowleri), all of which require design of specialized compounds that penetrate and spare cells in the CNS while effectively targeting pathogens of interest.

Haitao Guo, PhD

Hepatitis B Virus cccDNA Biosynthesis, Epigenetics, and Antiviral Development

Dr. Haitao Guo received his PhD in 2001 from Wuhan University, China. From 2002 to 2004, he received his postdoctoral training under supervision of Dr. Bill Mason in Fox Chase Cancer Center, where he started his research on hepatitis B virus (HBV). Then he joined Drexel University as a member of faculty for 9 years. In 2014, he joined Indiana University as an Associate Professor and became Full Professor in 2019. Since fall 2019, he is a Professor in **University of Pittsburgh** and Co-Leader of cancer virology program in UPMC Hillman Cancer Center. Dr. Guo's research is focused on HBV molecular biology, virus-host interaction, innate control of virus infection, and antiviral development. Dr. Guo has been serving as editor and/or editorial board member for major journals such as Antiviral Research, Journal of Medical Virology, Journal of Virology, Hepatology, etc., and he has served as a standing member of NIH Virology B Study Section (2017-2021). He is currently the Co-Chair of the Hepatitis B Special Interest Group for the American Association for the Study of Liver Diseases (AASLD), Co-Chair of Virology Working Group of the International Coalition to Eliminate Hepatitis B (ICE-HBV), and Member of the Scientific Advisory Council of International HBV Meeting. He has served as the co-organizer of the 2015 International HBV Meeting. Dr. Guo has published more than 100 papers and 3 patents. Dr. Guo has been awarded the Bruce Witte Fellow of Hepatitis B Foundation (2008-2014), IUSM Showalter Scholar (2017-2019), 2017 IUPUI Research Frontiers Trailblazer Award, 2019 IU Trustee's Teaching Award, 2020 UPMC Hillman Senior Faculty Fellow for Innovative Cancer Research, and he was elected to the American Academy of Microbiology (AAM) in 2022.

PLENARY SPEAKER
Edward Holmes, FAA, FRS

Virus Emergence at the Human-Animal Interface

Edward Holmes is an NHMRC Leadership Fellow and Professor of Virology in the School of Medical Sciences, **University of Sydney, Australia**, which he joined in 2012. Eddie received his undergraduate degree from the University of London (1986) and his PhD from the University of Cambridge (1990). Between 1993-2004 he held various positions at the University of Oxford, including University Lecturer in Evolutionary Biology and Fellow of New College. He was elected a Fellow of the Australian Academy of Science (FAA) in 2015 and of the Royal Society (FRS) in 2017. In 2020 he won the New South Wales Premier's Prize for Science and Engineering, and in 2021 he received the Australian Prime Minister's Prize for Science. He was recently awarded the Croonian Medal and Lecture 2024 by the Royal Society.

Tobias Lanz, MD

The B Cell Repertoire in Multiple Sclerosis Reveals Molecular Mimicry between EBV EBNA1 and GlialCAM

Tobias Lanz is an assistant professor at the Institute for Immunity, Transplantation, and Infection and the Division of Immunology and Rheumatology at **Stanford University**. His lab's research focuses on B cell biology in autoimmune and neuroimmunological diseases. He uses high-throughput screening technologies, and methods from structural and cell biology to identify new autoantigens and to understand how certain self-reactive B cells escape tolerance mechanisms. He is particularly interested in molecular mechanisms that explain the association between Epstein Barr Virus (EBV) and autoimmunity. Tobias went to medical school at the Eberhard Karls University in Tübingen, Germany and at the University College of London. He wrote his MD thesis at Dr. Michael Platten's laboratory at the Hertie Institute for Clinical Brain Research in Tübingen, Germany before joining Dr. Lawrence Steinman's neuroimmunological laboratory at Stanford as a research scholar. After medical school he pursued his scientific and clinical training at the German Cancer Research Center (DKFZ) and the Department of Neurology at the University Hospital in Heidelberg, Germany. In 2015 he joined Dr. William Robinson's lab at Stanford, where he investigated environmental triggers of autoimmunity, including viruses and milk consumption. In his most recent work, he characterized the B cell repertoire in the spinal fluid of patients with multiple sclerosis (MS) and identified molecular mimicry between EBV EBNA1 and the glial cellular adhesion molecule GlialCAM as a driver of neuroinflammation (Lanz et al., Nature, 2022). His long-term objective is to further understand how viruses contribute to or trigger autoimmunity and to develop next-generation targeted antiviral therapeutics to treat autoimmune diseases.

Alpha Lee, PhD

Accelerating Antiviral Discovery with Artificial Intelligence

Dr. Alpha Lee is the co-founder and Chief Scientific Officer of **PostEra**, and a faculty member at the University of Cambridge. He is also a co-Principal Investigator of the NIH-funded Antiviral Drug Discovery (AViDD) center "AI-driven Structure-Enabled Antiviral Platform". Alpha's research focuses on advancing machine learning technologies that accelerate medicinal chemistry for small molecule drug discovery. He was trained at Harvard University (Fulbright Scholar and George F. Carrier Fellow), and University of Oxford (DPhil). Alpha has been named by Forbes as 30 under 30 in Science and Healthcare in Europe.

Sharon Lewin, AO, FRACP, PhD

Towards and HIV Cure – Novel Approaches to Reduce and Control the Reservoir

Professor Sharon Lewin is an infectious diseases physician and basic scientist, who is internationally renowned for her research into all aspects of HIV disease and specifically in strategies to achieve an HIV cure. She received her medical degree and PhD from Monash University, Melbourne, Australia and post-doctoral training at Rockefeller University, New York, where she worked with Professor David Ho, Time Man of the Year in 1995 for his work in HIV treatments. She is the inaugural Director of the **Doherty Institute**, a joint venture of the **University of Melbourne** and Royal Melbourne Hospital and Melbourne Laureate Professor of Medicine at the University of Melbourne, Melbourne, Australia. She is also the inaugural director of the Cumming Global Centre for Pandemic Therapeutics, a new centre at the Doherty Institute established by a philanthropic gift of $250 million from Canadian philanthropist Geoff Cumming and $75 million from the Victorian government. She heads a laboratory of 25 scientists and clinicians working on basic and translational research and early phase clinical trials aimed at finding a cure for HIV. She has received continuous funding from the NHMRC since 1993 and from multiple international funding agencies including the National Institute for Health and the American Foundation for AIDS Research. In 2019, She was appointed an Officer of the Order of Australia (AO) in recognition of her distinguished service to medical research, and to education and clinical care, in the field of infectious diseases, particularly HIV and AIDS. She is the elected President of the International AIDS Society (2022-2024), the largest professional society representing people working in HIV medicine and has over 17,000 members.

Margaret Littlejohn, PhD, VIDRL

Advances Towards HBV Cure

Dr. Margaret Littlejohn is a senior medical scientist at the Victorian Infectious Diseases Reference Laboratory, and Honorary Fellow in the Department of Infectious Diseases, based at the **Peter Doherty Institute for Infection and Immunity** at the University of Melbourne. She is an expert in HBV molecular virology and the role of HBV genotypes in pathogenesis and treatment response and is currently leading a project funded by the mRNA Victoria Activation program to develop a new RNA-based therapy for chronic hepatitis B, using CRISPR technology.

She is also involved in a long term collaboration examining the molecular epidemiology of HBV in Indigenous Australian populations. This was the first comprehensive molecular analysis of the HBV detected in the Australian Indigenous population, discovering a unique HBV sub-genotype found exclusively in the Indigenous Australian population.

Hong Liu, PhD

Development of Broad-Spectrum Antiviral Drugs

Prof. Hong Liu is the Professor in **Shanghai Institute of Materia Medica**, and she has mainly focused on the medicinal chemistry, chemical biology, drug design and antiviral drug development. She has constructed a drug-like-compound library containing more than 400 structural scaffolds and more than 8000 compounds. Among them, 9 novel drug candidates were successfully licensed to Pharmaceutical Co., Ltd. In addition, 2 antiviral drug candidates, FB2001(Bofutrelvir, an anti-COVID-19 drug candidate) and Thioraviroc (an anti-HIV drug candidate), have completed Phase I and entered Phase II or Phase III clinical studies. Prof. Liu has published more than 450 academic papers as corresponding author in peer-review journals, such as Science Nature Chemical Reviews Cell Metabolism Nature Communications Angewandte Chemie International Edition Science Advances, etc., and these articles have been cited more than 18000 times by others. Prof. Liu has published 16 books as chapter author as well and obtained 137 patent certifications.

Sarah Londrigan, PhD

Identification of Novel Host Proteins that are Associated with Macrophage Control of Influenza A Virus Replication

Dr. Sarah Londrigan is a teaching and research academic in the Department of Microbiology and Immunology at **The University of Melbourne** in Australia. She is also Co-Lead of the Viral Infectious Diseases Theme at the **Doherty Institute** in Melbourne. Sarah leads a research program examining cellular responses to respiratory virus infection, including how macrophages control influenza A virus replication. Dr. Londrigan completed her PhD Research at Melbourne University, where she identified novel cell surface receptors for rotavirus entry during infection of host cells. Sarah's postdoctoral research at The Walter and Eliza Hall Institute in Australia involved creating immunomodulatory adenoviruses that generated local immunosuppression during islet transplantation to treat type I diabetes. Sarah's current research program is funded through competitively awarded National Health and Medical Research Council (NHMRC) grants. Her research program aims to understand the entry pathways of respiratory viruses into host cells, and how airway immune cells control virus replication. Specifically, these studies are focused on (i) identifying cell surface receptors and entry pathways for influenza and other respiratory viruses into airway macrophages, (ii) identifying host factors (including interferon stimulated genes) responsible for controlling respiratory virus replication and (iii) investigating novel antiviral strategies to control virus-induced respiratory disease. Sarah plays an active role in research related activities supporting virology, immunology and promoting women in science.

PLENARY SPEAKER
Jenny Low, MBBS, MPH

Bringing Antivirals to the Clinic: Challenges and Opportunities

Dr. Jenny Low is a senior consultant with the Department of Infectious Diseases in **Singapore General Hospital** and Professor at the Emerging Infectious Diseases Programme at **Duke NUS Medical School**. Concurrently, she is the co-director of the Viral Research and Experimental Medicine Centre@ SingHealth Duke-NUS (ViREMiCS) in the SingHealth Duke NUS AMC and deputy clinical and scientific director at the SingHealth Investigational Medicine Unit (IMU). Dr Low has been doing clinical research for more than 20 years. She has a long track record in conducting proof of concept and early phase clinical trials in acute viral diseases. She has tested several first-in-human therapeutics and biologics in humans including a therapeutic anti-yellow fever virus antibody that was published in the New England Journal of Medicine in 2020. During the COVID-19 pandemic, among her contributions, she led a clinical study that was among the first to detail the host response to severe COVID-19 which was published in Cell Host & Microbe in 2020. She also led the COVID-19 self-amplifying mRNA vaccine trial that was co-developed by Duke-NUS and Arcturus therapeutics among others. The vaccine is currently licensed for use in Vietnam and Japanese. Her current research focus is on early phase adaptive clinical trials of viral therapeutics and vaccine development as well as understanding the role of the early immune responses in modulating the outcome of infection or vaccination. She has been twice awarded the National Clinician Scientist Award in 2016 and 2019 for her research in host immune response to viral infections.

Lisa Ng, PhD

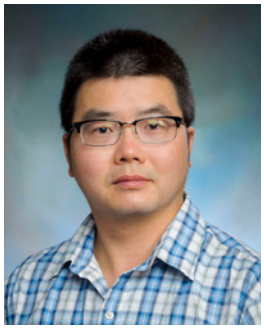
Cellular and Molecular Mechanisms of Arboviral Immunity

Prof. Lisa Ng is currently Executive Director at the **A*STAR Infectious Diseases Labs**. She also holds a joint appointment at the Biomedical Research Council, A*STAR as Executive Director. Lisa has been in the area of infectious diseases more over 25 years, where she has provided major contributions in the containment, prevention and treatment of epidemic viral infections including SARS (Severe Acute Respiratory Syndrome) and avian influenza H5N1 (bird flu). The current research interest of her group focuses on the immune responses of viruses that are epidemic or highly endemic in the tropical region. Over the years, these include chikungunya virus, dengue virus, Zika virus and other related alpha- and flavi-viruses, and more recently SARS-CoV-2. Her team has published in top tier scientific journals and made several key important findings to move the human immunology field forward in controlling viral infections. For her contributions, she was voted "Most Inspiring Woman" at the Great Women of Our Time Awards for Science and Technology in 2005, and was conferred the Junior Chamber International (JCI) "Ten Outstanding Young Persons of the World" Singapore 2013 Scientific and/or Technological Development Award. For her meritorious research and development efforts on Asia's infectious diseases, she was conferred the highly prestigious ASEAN (Association of South-East Asian Nations) "International Young Scientist and Technologist Award" in 2008. In recognition of her mentoring work for graduate students and scientists, Dr Ng received the A*STAR "Most Inspiring Mentor Award" in 2013. She was a recipient of the Public Administration Medal (Bronze) in 2016: the National COVID-19 Award (Silver) in 2022; and the Public Administration Medal (Silver) in 2023. She is a SNAS (Singapore National Academy of Sciences) 2022 fellow, and an elected member of the Henry Kunkel society for immunologists of human diseases.

Dan Watterson, PhD

Combating Emerging Henipaviruses

Professor Dan Watterson is a molecular and structural virologist from the **University of Queensland**, Brisbane, Australia. His research focuses on using structural information to design new vaccines and therapies for emerging viruses. Since the award of his PhD in 2012, he has published >90 papers, 51 in the last 3 years. He has co-invented two platform vaccine technologies that allow the production of viral antigens in the most protective conformation, and work across a wide range of viral families that cause current and potential future outbreaks. This work has lead to two clinical trials and ongoing support from CEPI for pandemic preparedness and rapid vaccine response. His lab is now repurposing these technologies to answer fundamental questions about viral antigen structure and how they interact with the immune system. He has led major discoveries across diverse viral families, including characterization of the first broadly protective anti-NS1 flavivirus antibody and resolving the highest resolution and first-in-class structures of important viruses including emerging henipaviruses with pandemic potential.



Xuping Xie, PhD

UNAPP: A Unique Academic-Industrial Partnership for Antiviral Research in Pandemic Preparedness

Xuping Xie is currently a Research Associate Professor within the Department of Biochemistry and Molecular Biology at the **University of Texas Medical Branch (UTMB)** in Galveston, United States. He has over 14 years of experience in both academia and industry, specializing in flavivirus and coronavirus research and countermeasure development. He earned his PhD degree in Biochemistry and Molecular Biology from the Chinese Academy of Sciences in 2012. During his PhD training, he pioneered the development of a preclinical NS4B inhibitor against the dengue virus. Driven by a passion for drug discovery, he pursued his postdoctoral training from 2013 to 2015 at the renowned Novartis Institute for Tropical Diseases in Singapore, focusing on anti-dengue drug development. In 2026, he joined UTMB and continued his research in flavivirus. He has revealed several important aspects of structural and nonstructural proteins in flavivirus replication, assembly, and pathogenesis. He also led the lab in the development of pivotal reverse genetic systems and platforms for countermeasure development during the Zika outbreak. When the COVID-19 pandemic began in 2020, he expanded his research into coronavirus. He developed the first peer-reviewed reverse genetic system for SARS-CoV-2, alongside high-throughput platforms for antiviral screening and neutralizing antibody testing. With a proven track record spanning both basic and translational research in the field of flavivirus and coronavirus, his lab stands at the forefront of pandemic preparedness. He is privileged to co-lead an interdisciplinary team from Novartis and the University of Texas Medical Branch (UNAPP) on drug discovery targeting coronaviruses, flaviviruses, and henipavirus.

Sunday, May 19, 2024

1:00 PM – 6:00 PM

Pre-Conference Symposium: An Australasian Virology Mini Symposium – Advancing Knowledge, Protecting People

GRIFFITH UNIVERSITY GOLD COAST CAMPUS

Separate registration required.

Click here for more info

Monday, May 20, 2024

12:00 PM – 1:45 PM

Special Event: Women in Science Roundtable

PHOENIX ROOM

Chaired by
Rhonda Carter

This event is at capacity.
If you have joined the waitlist, you will be notified if there are any openings.

2:00 PM – 4:15 PM

Opening Session and Plenary Session

NORFOLK BALLROOM

Chaired by
Luis Schang and **Kathie Seley-Radtke**

001. **Virus Emergence at the Human-Animal Interface**
Edward C. Holmes, FAA, FRS, *The University of Sydney, Australia, Sydney, NSW, Australia*

002. **Bringing Antivirals to the Clinic: Challenges and Opportunities**
Jenny G. Low, MBBS, MPH, *Singapore General Hospital, Duke NUS Medical School, Singapore, Singapore*

4:15 PM – 4:30 PM

Break

NORFOLK FOYER

4:30 PM – 5:30 PM

Gertrude Elion Memorial Award Lecture

NORFOLK BALLROOM

Chaired by
Luis Schang and **Kathie Seley-Radtke**

003. **My Battle Against Viruses**
Professor Johan Hendrik Neyts, *KU Leuven, Belgium*

5:30 PM – 6:30 PM

Opening Reception

RELISH GRILL AND BAR

Tuesday, May 21, 2024

8:30 AM – 9:15 AM

William Prusoff Memorial Award Lecture

NORFOLK BALLROOM

Chaired by
Luis Schang and **Kathie Seley-Radtke**

004. **Viral Hemorrhagic Fevers: Challenges and Gains of Animal Model Research for Pre-Clinical Vaccine and Antiviral Screening**
Jessica R. Spengler, D.V.M., Ph.D, M.P.H., *US Centers for Disease Control and Prevention, Atlanta, GA, United States*

9:15 AM – 10:00 AM

Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness

NORFOLK BALLROOM

Chaired by
John Bilello and **Subash Vasudevan**

005. **Oral Pharmacokinetics and Efficacy of Modified Oral Lipid RVn Prodrugs Against SARS-CoV-2 in Mice**
Aaron F. Carlin, M.D. Ph.D, *Department of Pathology and Medicine, University of California at San Diego, La Jolla, California, United States*

006. **Towards a Novel Host-Targeted Anti-Infective Strategy Against COVID-19 and Other Acute Respiratory Viral Diseases**
Stephan Ludwig, PhD, *Institute of Virology, Muenster, Germany*

007. **Exploring Viral RNA Methyltransferases for Antiviral Drug Design**
Radim Nencka, Ph.D., *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

008. **Targeting Host Kinases to Identify Novel Broad-Spectrum Antiviral Strategies**
Valeria Cagno, Ph.D., *Institute of Microbiology, Lausanne University Hospital, University of Lausanne, Lausanne, Vaud, Switzerland*

10:00 AM – 11:00 AM

NORFOLK BALLROOM

PechaKucha Competition

Chaired by
Kathie Seley-Radtke

11:00 AM – 11:15 AM

Break

NORFOLK FOYER

11:15 AM – 12:15 PM

Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness *(continued)*

NORFOLK BALLROOM

Chaired by
John Bilello and **Subash Vasudevan**

009. **UNAPP: A Unique Academic-Industrial Partnership for Antiviral Research in Pandemic Preparedness**
Xuping Xie, Ph.D., *UTMB, Galveston, Texas, United States*

010. **Molnupiravir Extends its Broader Spectrum of Activity to Human Norovirus and Rotavirus in 3D Human Intestinal Enteroids**
Nanci Santos-Ferreira, *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

011. **Targeting mpox Virus Resolvase (Mpr): In Vitro Assay Development and Inhibitors**
Zhengqiang (ZQ) Wang, Ph.D., *Center for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, United States*

012. **Potential Genetic Determinants Affecting Virulence of Heartland Bandavirus Infection in Mice and Therapeutic Intervention with the Ribonucleoside Analog, EIDD-2749**
Jonna B. Westover, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

12:15 PM – 1:45 PM

Lunch

(included for all conference registrants)

RELISH GRILL AND BAR

12:30 PM – 1:30 PM

Special Event: Career Development Roundtable

PHOENIX ROOM

Chaired by
Leen Delang

This event is at capacity.
If you have joined the waitlist, you will be notified if there are any openings.

1:45 PM – 2:30 PM

Diversity in Science and Excellence Award Lecture

NORFOLK BALLROOM

Chaired by
Victor Garcia-Martinez and **Luis Schang**

013. **Chasing an HIV Cure: The Intersection of Biological Sex and Latency Reversal**
Nancie Marie Archin, PhD, *The University of North Carolina UNC HIV Cure Center, Chapel Hill, North Carolina, United States*

2:30 PM – 3:30 PM

Coronaviruses, Influenza, RSV, and Other Respiratory Viruses

NORFOLK BALLROOM

Chaired by
Rhonda Cardin and **Tim Sheahan**

014. **Drug Discovery Efforts Towards Human Metapneumovirus**
Larissa Dirr, Ph.D., *Institute for Glycomics, Griffith University, Australia*

021. **Nanobodies Against COVID-19 and Other Emerging Viruses**
Fang Li, Ph.D., *University of Minnesota, Minneapolis, MN, United States*

016. **Activating the RIG-I Pathway by RNA Agonists or Small Molecule Drugs Triggers Innate Immune Programming to Control Infection by Influenza A virus and SARS-COV2**
Amina Negash, Ph.D, *Center for Innate Immunity and Immune Disease, Department of Immunology, University of Washington, Seattle, Washington, United States*

017. **Understanding and Inhibiting SARS-CoV-2 NiRAN Domain Catalytic Activities Through Structural Studies and Large-Scale Docking**
Gabriel Small, *Laboratory of Molecular Biophysics at The Rockefeller University, New York, NY, United States*

3:30 PM – 3:45 PM

Break

NORFOLK FOYER

3:45 PM – 5:00 PM

Coronaviruses, Influenza, RSV, and Other Respiratory Viruses *(continued)*

NORFOLK BALLROOM

Chaired by
Rhonda Cardin and **Tim Sheahan**

018. **Combating Emerging Henipaviruses**
Daniel Watterson, PhD, *University of Queensland, Brisbane, Australia*

019. **Design of SARS-CoV-2 Papain-like Protease Inhibitor with Antiviral Efficacy in a Mouse Model**
Jun Wang, *Rutgers, The State University of New Jersey, Piscataway, New Jersey, United States*

020. **Identification of Adenosine Analogues that Inhibit the N7 Methyltransferase Activity of SARS-CoV-2**
Adrien Delpal, Ph.D, *AFMB, Marseille, France*

015. **Identification of Novel Small-Molecule Inhibitors of SARS-CoV-2 by Chemical Genetics**
Shuofeng Yuan, *The University of Hong Kong, Hong Kong (SAR China)*

022. **Intranasal Administration of a Live Attenuated Vaccine Derived from NSP16-deficient SARS-CoV-2 Confers Sterilizing Immunity in Rodent Models**
Zi-Wei Ye, Ph.D., *The University of Hong Kong, Hong Kong, Hong Kong (SAR China)*

5:00 PM – 7:00 PM

Poster Session 1

MONACO AND SIFU

Light food and beverages provided.

5:00 PM – 6:00 PM
ODD numbered posters

6:00 PM – 7:00 PM
EVEN numbered posters

Wednesday, May 22, 2024

8:30 AM – 9:10 AM

Chronic, Latent, and Persistent Viruses - Retroviruses and Herpesviruses

NORFOLK BALLROOM

Chaired by
Joy Feng and **Zlatko Janeba**

023. **Development of Broad-Spectrum Antiviral Drugs**
Hong Liu, PhD, *Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China*

024. **Some Capsid/Core Assembly Modulators (CAMs) Can Induce an Inhibition of HBV RNA Biogenesis**
David Durantel, Ph.D., *Equipe Hepvir, CIRI, Inserm U1111, CNRS UMR5308, ENS-Lyon, UCBL1, Lyon, France*

9:10 AM – 10:30 AM

Late-breaking Oral Presentations and Hepatotropic and GI Viruses

NORFOLK BALLROOM

Chaired by
Kara Carter and **David Durantel**

080. **Design and Synthesis of Clickable Photoaffinity Probes for Binding Site Identification on Yellow Fever Virus NS4B Target Built upon a Benzodiazepine Antiviral**
Yanming Du, *Baruch S. Blumberg Institute, Doylestown, PA, United States*

081. **Establishment of the First High-Throughput Screening Assay for Rhinovirus C Antiviral Drug Discovery**
Erion Lyoo, Ph.D., *KU Leuven, Leuven, Belgium*

082. **Suppression of Hepatitis B Virus Replication and Protein Expression Using CRISPR-Cas13b – Pre-clinical Investigations of a New Antiviral Approach**
Margaret Littlejohn, Ph.D, VIDRL, *Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity, Australia*

083. **The CD8+T Cells Response is Sufficient for Protection with a CCHFV M-segment Based DNA Vaccine and GP38 Enhances Vaccine Immunogenicity**
Aura Garrison, Ph.D., *USAMRIID, Frederick, United States*

084. **Unveiling Host-cell Glycosylation Changes Upon Parainfluenza Virus Infection**
Plabon Kumar Das, M. S., *Institute for Glycomics, Griffith University, Gold Coast Campus, Qld, Australia, Gold Coast Australia, Queensland, Australia*

029. **Advances Toward HBV Cure**
Margaret Littlejohn, Ph.D, VIDRL, *Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity, Australia*

10:30 AM – 10:45 AM

Break

NORFOLK FOYER

10:45 AM – 12:15 PM

Arboviruses

NORFOLK BALLROOM

Chaired by
Lara Herrero and **Dahai Luo**

030. **Beyond Retroviruses: Restriction of Flavivirus Replication by TRIM5α**
Sonja Marie Best, PhD, *National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, United States*

031. **Liver-targeted Therapeutic siRNAs Against Highly Conserved Yellow Fever Virus Genomic Sequences Effectively Limit Infection and Mortality in a Hamster Model**
Justin G. Julander, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

032. **Ingestion of the Antiviral Drug JNJ-A07 by Mosquitoes During Blood-feeding Significantly Reduced Dengue Virus Transmission**
Ana Lucia Rosales Rosas, *KU Leuven, Leuven, Belgium*

033. **Structural Basis of Dengue and Zika Virus NS1 Multimerization and Antibody Recognition**
Alvin Bing Liang Chew, *Nanyang Technological University, Singapore*

034. **Identification of mRNA Processing Machinery as Druggable Host Factor Targets for Dengue Virus Infection**
Min Jie Alvin Tan, Ph. D., *Duke-NUS Medical School, Singapore*

035. **Discovery of Pan-flavivirus Protease Inhibitors**
Christoph Nitsche, *Australian National University, Canberra, ACT, Australia*

036. **Combination Therapy of Approved Drugs Potentiates Broad-spectrum Antiviral Activity Against Alphaviruses in Human Skin Fibroblasts And Mice**
Leen Delang, PhD, *KU Leuven, Leuven, Belgium*

12:15 PM – 2:15 PM

Poster Session 2

MONACO AND SIFU

Lunch provided.

12:15 PM – 1:15 PM
EVEN numbered posters

1:15 PM – 2:15 PM
ODD numbered posters

Thursday, May 23, 2024

8:30 AM – 9:20 AM

Machine Learning and Computational Approaches for Antiviral Research

NORFOLK BALLROOM

Chaired by
Alpha Lee and **Joshua Schiffer**

037. **Accelerating Antiviral Discovery with Artificial Intelligence**
Alpha Lee, *PostEra, Cambridge, MA, United States*

038. **Atomistic Model of the Coronavirus nsp3/nsp4 Double Membrane Vesicle Pore**
Jason K. Perry, Ph.D, *Gilead Sciences, Inc., Foster City, CA, United States*

039. **Antiviral Therapy Optimization for SARS-CoV2: A Mathematical Modeling Approach**
Shadi Sadat Esmaeili-Wellman, Ph.D., *Fred Hutch Cancer Center, Seattle, WA, United States*

9:20 AM – 10:20 AM

Coronaviruses, Influenza, RSV, and Other Respiratory Viruses

NORFOLK BALLROOM

Chaired by
Larissa Dirr and **Mark von Itzstein**

040. **Click Chemistry-based Rapid Identification and Crystallographic Studies of Novel 1,2,3-Triazole-bearing Diazabicyclooctane Derivatives as Non-Covalent SARS-CoV-2 Mpro Inhibitors with Potent Antiviral Activity and Improved Drug-resistance Profile**
Peng Zhan, Ph.D., *Shandong University, Jinan, China*

041. **Identification of Small-Molecule Inhibitors of Coronaviruses by Targeting Protein-Protein Interactions in RNA-Dependent RNA Polymerase Complex**
Jeremy Blavier, MS, *Viral Interactomes Laboratory, GIGA Institute, University of Liege, Liege, Belgium*

042. **A CRISPR/Cas9 Genetically Engineered Organoid Biobank To Study Coronavirus Host Factors**
Mart Matthias Lamers, *Duke-NUS, Singapore*

10:20 AM – 10:35 AM

Break

NORFOLK FOYER

10:35 AM – 11:25 AM

Coronaviruses, Influenza, RSV, and Other Respiratory Viruses *(continued)*

NORFOLK BALLROOM

Chaired by
Larissa Dirr and **Mark von Itzstein**

043. **Identification of Novel Host Proteins that are Associated with Macrophage Control of Influenza A Virus Replication**
Sarah L. Londrigan, PhD, *Department of Microbiology and Immunology, The University of Melbourne, Peter Doherty Institute, Melbourne, Victoria, Australia*

044. **A Mouse Model of Human Parainfluenza Virus Type 3 Infection to Study Prophylactic and Therapeutic Modalities**
Yuxia Lin, Ph.D, *KU Leuven, Leuven, Belgium*

045. **Engineering Protease-resistant Peptides to Inhibit Human Parainfluenza Viral Respiratory Infection**
Anne Moscona, M.D., *Columbia University Vagelos College of Physicians and Surgeons, New York, NY, United States*

046. **Aptamer-based Glycoprotein Broad-spectrum Blocking Strategy Inhibits Respiratory Syncytial Virus Infection**
Ge Yang, *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical, Beijing, China*

047. **Inhibition of Rhinovirus Infection in Differentiated Primary Human Bronchial Epithelial Cells by Nanoparticle-Encapsulated Small Interfering RNA**
Nathan Bryant, *University of Newcastle, Newcastle, NSW, Australia*

048. **Development of Small Molecule Entry Inhibitors as Novel Therapeutics against Influenza Viruses**
Lijun Rong, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States; Chicago BioSolutions, Inc., Chicago, Illinois, United States*

11:25 AM – 12:00 PM

Chronic, Latent, and Persistent Viruses – Retroviruses and Herpesviruses

NORFOLK BALLROOM

Chaired by
Gerald Kleymann and **Jennifer Moffat**

050. **The B Cell Repertoire in Multiple Sclerosis Reveals Molecular Mimicry between EBV EBNA1 and GlialCAM**
Tobias V. Lanz, MD, *Stanford University, Stanford, CA, United States*

12:00 PM – 12:15 PM

ISAR Annual Business Meeting

NORFOLK BALLROOM

PRESIDENT: **Kathie Seley-Radtke**

TREASURER: **Brian Gowen**

SECRETARY: **Brian Gowen** on behalf of **Jinhong Chang**

12:15 PM – 1:45 PM

Lunch

(included for all conference registrants)

RELISH GRILL AND BAR

1:45 PM – 2:30 PM

Women in Science and Excellence Award Lecture

NORFOLK BALLROOM

Chaired by
Rhonda Cardin and **Kathie Seley-Radtke**

051. **From Target to Treatment**
Judith Breuer, MD, FRCPath, FMedSci, *University College London and Great Ormond Street Hospitals, London, England, United Kingdom*

2:30 PM – 3:30 PM

Arboviruses

NORFOLK BALLROOM

Chaired by
Lara Herrero and **Dahai Luo**

052. **Medicinal Chemistry Optimization and Therapeutic Efficacy of 2-Pyrrolidinoquinazolinones in Lethal Murine Models of Venezuelan and Eastern Equine Encephalitis Viruses**
Jennifer E. Golden, Ph.D., *Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States*

053. Now virtual poster
Design, Synthesis, and Lead Optimisation of Piperazinyl-Pyrimidine Analogues as Potent Small Molecules Inhibitors of Chikungunya Virus
Verena Battisti, Ph.D, *University of Vienna, Vienna, Vienna, Austria*

054. **Suicidal Capsid Protease from O'nyong'nyong Virus: Unveiling the Inhibitory Potential of Indole Derivatives**
Yuliya Chykunova, *Virogenetics Laboratory of Virology, Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland*

055. **Treatment with 6MMPr Potentiates the Activity of Favipiravir in a Hamster Model of Yellow Fever**
Justin G. Julander, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

3:30 PM – 3:45 PM

Break

NORFOLK FOYER

3:45 PM – 4:45 PM

Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness

NORFOLK BALLROOM

Chaired by
John Bilello and **Subash Vasudevan**

056. **Cellular and Molecular Mechanisms of Arboviral Immunity**
Lisa Ng, Ph.D., *A*STAR Infectious Diseases Labs, Singapore*

057. **Development and Mechanism of Novel Diphyllin Derivatives Against Ebola Virus Infection**
Patrick Keiser, PhD Candidate, *NEIDL, Department of Virology, Immunology, and Microbiology, Boston University, Boston, MA, United States*

058. **Identification and Evaluation of Novel Lassa Virus Entry Inhibitors Using Computational Counter Screening And Chemical Informatics**
Brianna Close, *Boston University, Boston, Massachusetts, United States*

059. **Identification of a Macrocyclic Compound Targeting the Lassa Virus Polymerase**
Mike J. Flint, Ph.D., *Centers for Disease Control and Prevention, Atlanta, GA, United States*

4:45 PM – 5:00 PM

Break

NORFOLK FOYER

5:00 PM – 6:00 PM

Broad Spectrum Antiviral Drugs, Non-respiratory Biothreat Viruses, and Pandemic Preparedness *(continued)*

NORFOLK BALLROOM

Chaired by
John Bilello and **Subash Vasudevan**

060. **Tribbles Pseudokinase 3 Promotes Enterovirus A71 Infection via Dual Mechanisms**
Huiqiang Wang, *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, China*

061. **Generation and Optimization of Bangladesh and Malaysian Recombinant Reporter Nipah Viruses for Antiviral Screening in vitro and Disease Modeling in vivo**
Michael K. Lo, Ph.D, *US Centers for Disease Control and Prevention, Atlanta, GA, United States*

062. **Intranasal Route to Immunity: Single Dose Mucosal Delivery of Viral Replicon Particle Vaccine Protects Uniformly Against Lethal Nipah Virus Challenge In African Green Monkeys**
Stephen R. Welch, *US Centers for Disease Control and Prevention, Atlanta, United States*

063. **Evaluation of Small Molecules as Promising Broad-Spectrum Anti-Filoviral Agents**
Jazmin Galvan Achi, *University of Illinois Chicago, Chicago, Illinois, United States*

064. **Small Molecule Antiviral Candidates for Rift Valley Fever**
Wenjun Ma, *University of Missouri, Columbia, Missouri, United States*

065. **In Silico Tools for Antiviral Research and Future Pandemic Forecasting**
Eugene Muratov, Ph.D., *University of North Carolina, Chapel Hill, North Carolina, United States*

7:00 PM – 10:00 PM

Closing Event

NORFOLK BALLROOM & POOLSIDE

Friday, May 24, 2024

9:00 AM – 10:20 AM

Hepatotropic and GI Viruses

NORFOLK BALLROOM

Chaired by
Kara Carter and **David Durantel**

066. **Hepatitis B Virus cccDNA Biosynthesis, Epigenetics, and Antiviral Development**
Haitao Guo, Ph.D, *University of Pittsburgh, Pittsburgh, Pennsylvania, United States*

067. **Discovery of a Pan-genotype Hepatitis E Virus Replication Inhibitor Exerting Potent in vivo Efficacy**
Suzanne Kaptein, Ph.D., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

068. **Functional Evaluation and Mode of Action of a Novel Non-nucleoside Drug Inhibiting the Replication of Hepatitis Delta Virus**
David Durantel, Ph.D., *Equipe Hepvir, CIRI, Inserm U1111, CNRS UMR5308, ENS-Lyon, UCBL1, Lyon, France*

069. **Discovery of First-in-Class Hydrophobic Tagging (HyT)-based Degraders of HBV Core Protein**
Shujing Xu, Ph.D., *Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), Jinan, Shandong Province, China*

070. **Proof of Concept: Exploring the Therapeutic Potential of G3BP1 Targeted Degradation Against Norovirus Infection**
Liliana Echavarria Consuegra, Dr., *University of Cambridge, Cambridge, United Kingdom*

071. **Protease Inhibitor Activity Varies Between Genogroup I and Genogroup II Noroviruses**
Alice M. McSweeney, *Otago University, New Zealand*

10:20 AM – 10:45 AM

Break

NORFOLK FOYER

10:45 AM – 11:15 AM

Shotgun Presentations

NORFOLK BALLROOM

Chaired by
Two Trainees TBD

11:15 AM – 12:30 PM

Chronic, Latent, and Persistent Viruses – Retroviruses and Herpesviruses

NORFOLK BALLROOM

Chaired by
Joy Feng, Zlatko Janeba, Jennifer Moffat, and **Gerald Kleymann**

072. **Towards an HIV Cure: Novel Approaches to Reduce and Control the Reservoir**
Sharon R. Lewin, Ph.D., *Department of Infectious Diseases, University of Melbourne, Doherty Institute, Melbourne, VIC, Australia*

073. **Antivirals Targeting the Conserved HIV-1 TIM-TAM Riboswitch Specifically Reactivate HIV-1 from Latency through Modulating Viral RNA-biology**
Damian FJ Purcell, Ph.D., *Department of Microbiology and Immunology, University of Melbourne, Doherty Institute, Melbourne, VIC, Australia*

074. **Mechanisms of HIV-1 Hypersensitivity to Islatravir (4'-ethynyl-2-fluoro-2'-deoxyadeonsine (EFdA))**
Alexa A. Snyder, *Emory University School of Medicine, Atlanta, Georgia, United States*

075. **HSV-1 Latency is Established in Human Neurons in which Viral Genes are Expressed and Viral DNA is Replicated during the Acute Infection**
Arryn Owens, *Cornell University, Ithaca, NY, United States*

076. **Evaluation and Pharmacokinetics of the POM-L-BHDU-MP Prodrug Against Varicella Zoster Virus and Herpes Simplex Virus 1 in vivo**
Jennifer F. Moffat, PhD, *SUNY Upstate Medical University, Syracuse, NY, United States*

*For full list of authors and abstract details, please go to **Abstracts** section.*

005.* **Oral Pharmacokinetics and Efficacy of Modified Oral Lipid RVn Prodrugs Against SARS-CoV-2 in Mice**
Aaron F. Carlin, M.D. Ph.D, *Department of Pathology and Medicine, University of California at San Diego, La Jolla, California, United States*

010.* **Molnupiravir Extends its Broader Spectrum of Activity to Human Norovirus and Rotavirus in 3D Human Intestinal Enteroids**
Nanci Santos-Ferreira, *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

017.* **Understanding and Inhibiting SARS-CoV-2 NiRAN Domain Catalytic Activities Through Structural Studies and Large-Scale Docking**
Gabriel Small, *Laboratory of Molecular Biophysics at The Rockefeller University, New York, NY, United States*

020.* **Identification of Adenosine Analogues that Inhibit the N7 Methyltransferase Activity of SARS-CoV-2**
Adrien Delpal, Ph.D, *AFMB, Marseille, France*

032.* **Ingestion of the antiviral drug JNJ-A07 by mosquitoes during blood-feeding significantly reduced dengue virus transmission**
Ana Lucia Rosales Rosas, *KU Leuven, Leuven, Belgium*

033.* **Structural basis of Dengue and Zika virus NS1 multimerization and antibody recognition**
Alvin Bing Liang Chew, *Nanyang Technological University, Singapore*

039.* **Antiviral therapy optimization for SARS-CoV2: a mathematical modeling approach**
Shadi Sadat Esmaeili-Wellman, Ph.D., *Fred Hutch Cancer Center, Seattle, WA, United States*

040.* **Click Chemistry-based Rapid Identification and Crystallographic Studies of Novel 1,2,3-Triazole-bearing Diazabicyclooctane Derivatives as Non-Covalent SARS-CoV-2 Mpro Inhibitors with Potent Antiviral Activity and Improved Drug-resistance Profile**
Peng Zhan, Ph.D., *Shandong University, Jinan, China*

048.* **Development of Small Molecule Entry Inhibitors as Novel Therapeutics against Influenza Viruses**
Lijun Rong, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States; Chicago BioSolutions, Inc., Chicago, Illinois, United States*

060.* **Tribbles Pseudokinase 3 Promotes Enterovirus A71 Infection via Dual Mechanisms**
Huiqiang Wang, *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, China*

062.* **Intranasal route to immunity: single dose mucosal delivery of viral replicon particle vaccine protects uniformly against lethal Nipah virus challenge in African green monkeys**
Stephen R. Welch, *US Centers for Disease Control and Prevention, Atlanta, United States*

063.* **Evaluation of Small Molecules as Promising Broad-Spectrum Anti-Filoviral Agents**
Jazmin Galvan Achi, *University of Illinois Chicago, Chicago, Illinois, United States*

**Also presenting a short oral presentation*

069* **Discovery of First-in-Class Hydrophobic Tagging (HyT)-based Degraders of HBV Core Protein**
Shujing Xu, Ph.D., *Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), Jinan, Shandong Province, China*

070* **Proof of Concept: Exploring the Therapeutic Potential of G3BP1 Targeted Degradation Against Norovirus Infection**
Liliana Echavarria Consuegra, Dr., *University of Cambridge, Cambridge, United Kingdom*

075* **HSV-1 Latency is Established in Human Neurons in which Viral Genes are Expressed and Viral DNA is Replicated during the Acute Infection**
Arryn Owens, *Cornell University, Ithaca, NY, United States*

076* **Evaluation and Pharmacokinetics of the POM-L-BHDU-MP Prodrug Against Varicella Zoster Virus and Herpes Simplex Virus 1 in vivo**
Jennifer F. Moffat, PhD, *SUNY Upstate Medical University, Syracuse, NY, United States*

100. **Ross River virus upregulates chemokine (c-c motif) ligand 5, enhancing metalloproteinase expression for virus-induced extracellular matrix degradation**
Wesley Freppel, Ph.D., *Institute for Glycomics, Griffith University, Southport, QLD, Australia*

101. **Utilizing National Health Insurance Research Database in Taiwan Searches the Potential Traditional Chinese Medicines as Antiviral for Dengue Virus Infection**
Yi-Jung Ho, Ph.D., *National Defense Medical Center, Taipei, Taiwan, Province of China*

102. **The Role of Fibronectin in the Pathogenesis of Mosquito-borne Viral Disease**
Yong Qian Koo, *Institute for Glycomics, Griffith University, Southport, Australia*

103. **Old world alphaviruses induce rapid and strong neuroinflammation in primary human astrocytes**
Penny A. Rudd, Ph.D., *Institute for Glycomics, Griffith University, Southport, Queensland, Australia*

104. **Identification of anisomycin as a novel inhibitor of Chikungunya virus**
Youichi Suzuki, Ph.D., *Osaka Medical and Pharmaceutical University, Takatsuki, Japan*

105. **Unlocking the Flaviviral Blueprint: Leveraging Flaviviral Regulation of Selective Autophagy for Therapeutic Intervention**
Sophie van Leur, Ph.D., *Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom*

106. **Carbazole to indolazepinone scaffold morphing generates potent cell-active Dengue antivirals**
Gerry Rassias, Ph.D. , Prof, *University of Patras, Patra, Achaia, Greece*

107. **Niosomal and poly lactic-co-glycolic acid nanoparticles loaded with cannabidiol as an antiviral strategy against Zika virus**
Agostina Belén Marquez, M.S., *IQUIBICEN, Universidad de Buenos Aires- Consejo Nacional de Investigaciones Científicas y Técnicas, Ciudad Autónoma de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*

108. **Preclinical evaluation of insect-specific virus platform vaccines (ISVac) for Japanese encephalitis virus and Chikungunya virus in PC3 mouse models**
Daniel Rawle, *QIMR Berghofer Medical Research Institute, Herston, QLD, Australia*

**Also presenting a short oral presentation*

109. **Adjusting susceptibilities of C57BL/6 mice to flaviviruses for evaluation of antiviral drugs by altering the levels of interferon alpha/beta receptor function**
Venkatraman Siddharthan, Ph.D., *Utah State University, Institute for Antiviral Research, Logan, UT, United States*

110. **Development of messenger RNA vaccines targeting dengue virus non-structural proteins**
Satoru Watanabe, Ph.D., *Duke-NUS Medical School, Singapore*

111. **Serotype-Specific Regulation of Dengue Virus NS5 Protein Subcellular Localization**
Colin Cheng, PhD, *Charles Sturt University, Wagga Wagga, NSW, Australia*

115V. **A Yellow Fever Virus NS4B Inhibitor sequentially Inhibits Viral RNA Synthesis and Activates Double-Stranded RNA Reponses to Accelerate Apoptosis of Infected Cells**
Fuxuan Wang, *Baruch S. Blumberg Institute, Doylestown, PA, United States*

200. **Meta-analysis of clinical and virological data in the Syrian hamster model of Nipah virus disease to support translation to human disease and utility for antiviral screening**
Katherine A. Davies, Ph.D, *US Centers for Disease Control and Prevention, Atlanta, GA, United States*

201. **Discovery of Small Molecule Inhibitors against Henipaviruses**
Lijun Rong, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States*

202. **Antiviral activity of pyrophosphate analogues against arenavirus infection**
Johanna Briyith Diaz Sierra, Pd.D. Candidate, *Laboratorio de Virologia, FCEN, University of Buenos Aires, Argentina, Buenos Aires, Argentina*

203. **N-substituted Pyrrole-based Heterocycles as Broad-spectrum Filoviral Entry Inhibitors**
Destiny Durante, *University of Illinois Chicago, Chicago, United States*

204. **Design, synthesis, and biological evaluation of β-L-thymidine, β-L-2'-deoxycytidine, and other L-nucleoside reverse fleximer analogues**
Christianna Kutz, *University of Maryland, Baltimore County, Baltimore, Maryland, United States*

205. **Identification and characterization of small molecule inhibitors of SARS-CoV-2 RNA dependent RNA polymerase by targeting the NSP8-NSP12 interface**
Hongmin Li, *The University of Arizona, Tucson, Arizona, United States*

206. **Metformin Inhibits Replication of EV-A71 and CVA16 by Multiple Mechanisms**
Yuhuan Li, *Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China*

207. **Defective interfering RNA induces innate immunity and broad-spectrum antiviral activity**
Min-Hsuan Lin, Ph.D., *QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia*

208. **Plitidepsin broadly inhibits protein synthesis of distant viruses while reprograming the translational cellular landscape as a homeostatic response**
Elisa Molina Molina, *IrsiCaixa AIDS Research Institute, Can Ruti Campus, Badalona, Spain*

209. **Broad-spectrum antiviral potential of PI3K inhibitors**
Natalie E. Netzler, PhD, *University of Auckland, Maurice Wilkins Centre, Auckland, New Zealand*

210. **Evaluation of Potency and Metabolic Stability of Diphyllin-Derived Vacuolar-ATPase Inhibitors**
Laura M. Sanford, PhD Candidate, *BMCMP Department, Collage of Pharmacy, Purdue University, West Lafayette, Indiana, United States*

211. **Curcumin affects early and late steps of Junin virus multiplication in cell cultures** **Mariel Wagner, Ph.D. Seeking**, *University of Buenos Aires, Buenos Aires, Argentina*

212. **Targeting SARS-CoV-2 Nsp14 Methyltransferase: From In Silico Design to Nanomolar Inhibitors**
Hugo Kocek, *IOCB Prague; UCT Prague, Prague, Czech Republic*

213. **Antiviral activities of two nucleos(t)ide analogs against orthopoxviruses**
Zhilong Yang, Ph.D., *Texas A&M University, College Station, TX, United States*

214. **Removed (duplicate of 216)**

215. **The Extracellular Matrix Multi-Tissue Platform (MTP), Has Broad-Spectrum Antiviral Activity and Prevents Varicella Zoster Virus Spread in Cells and Human Skin**
Jennifer F. Moffat, PhD, *SUNY Upstate Medical University, Syracuse, NY, United States*

216. **Therapeutic efficacy of EIDD-2947 against poliovirus and coxsackievirus B3 in mice**
Venkatraman Siddharthan, Ph.D., *Utah State University, Institute for Antiviral Research, Logan, Utah, United States*

217. **GS-7682, a Novel Prodrug of a 4′-CN-4-Aza-7,9-Dideazaadenosine C Nucleoside with Broad-Spectrum Activity and Efficacy in RSV-infected African Green Monkeys**
John P. Bilello, Ph.D, *Gilead Sciences, Inc., Foster City, California, United States*

218. **Novel Virucidal Synthetic Polymers Targeting Enveloped Viruses**
Hylemariam Mengist, *The University of Queensland, Brisbane, Australia*

219. **Nasodine (0.5% PVP-I) Reduces SARS-CoV-2 Titers in COVID-19 Patients: a Phase II Trial**
Simon P. Tucker, PhD, *Firebrick Pharma Limited, Melbourne, VIC, Australia*

220. **Development of an antiviral assay in a specialized 3D liver tissue model in normal human-derived liver epithelial cells (EpiLiver™ LIV-100)**
Kie Hoon Jung, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

221. **Modifying Potent Broad-Spectrum Antiviral Imino-C-Nucleosides to Yield New Antiviral Leads**
Lawrence D. Harris, DPhil, *Ferrier Research Institute, Victoria University of Wellington, Wellington, New Zealand*

222. **VERAtide: the universal tool to enhance the efficacy of neutralizing antibodies**
Young-Do Kwon, Ph.D., *DaehanNupharm Co. Ltd, Seongnam-si, Gyeonggi-do, Republic of Korea*

223. **A Biocompatible, Virucidal Zwitterionic Antiviral Polymer Reduces Chikungunya Virus Infection in Murine Models**
Shannan-Leigh Macleod, *University of Manchester; and Agency for Science, Technology and Research (A*STAR), Singapore, Singapore*

224. **LHF-535 and favipiravir synergize to protect against experimental Junín virus infection and disease**
Brian B. Gowen, Ph.D., *Utah State University, Logan, Utah, United States*

225. **In vivo Evaluation of the Antiviral Efficacy of Subcutaneous Nafamostat Formulated with Glycyrrhizic Acid against COVID19**
Ju Hwan Jeong, Ph.D., *College of Medicine and Medical Research Institute, Chungbuk National University, ChengJu-si, Chungcheongbuk-do, Republic of Korea*

226. **Development of a multiplex screening assay for identifying novel antiviral targets involved in ebolavirus proteolysis**
Graham Simmons, Ph.D., *Vitalant Research Institute, San Francisco, California, United States*

227. **From Mystery to Mastery: Merkel cell polyomavirus Research and the Promise of siRNA Genomic Silencing**
Trairong Chokwassanakakulkit, Ph.D., *School of Pharmacy & Medical Sciences, Griffith University, Southport, Queensland, Australia*

228. **Nucleocapsid- and Glycoprotein 38 targeting monoclonal antibodies protect mice against Crimean-Congo hemorrhagic fever virus**
Joseph Golden, Ph.D., *USAMRIID, Ft. Detrick, Maryland, United States*

230V. **The Design, Synthesis, and Antiviral Evaluation of a Series of Flex-2'-deoxy-2'-fluoro-2'-methyl Nucleos(t)ide Analogues**
Charles Waters, *University of Maryland Baltimore County, Baltimore, MD, United States*

250V. **Preclinical Services available through NIAID's Division of Microbiology & Infectious Diseases (DMID)**
Fayna Diaz San Segundo, DVM, PhD, *NIIH-NIAID-DMID, Rockville, MD, United States*

251V. **Withdrawn**

252V. **Establishment of a lethal model of Nipah virus infection through transient immunosuppression of wild-type mice**
Teresa E. Sorvillo, Ph.D, *US Centers for Disease Control and Prevention, Atlanta, GA, United States*

254V. **Targeting Flaviviruses Replication by 5-Aminotriazole-amide Inhibitors**
Corinne E. Augelli-Szafran, *Southern Research, Birmingham, Alabama, United States*

255V. **Potent antiviral activity of Plitidepsin against Ebolavirus**
Rafael Delgado, MD, PhD, *Instituto de Investigación Hospital Universitario 12 de Octubre, Madrid, Spain*

256V. **Diaryl Ethers in Double Combinations with Pocapavir against Poliovirus 1 and Coxsackievirus B4**
Adelina Stoyanova, *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

300. **SARS-CoV-2 PLpro Inhibitors for COVID-19 Therapy**
Jazmin Galvan Achi, *University of Illinois Chicago, Chicago, IL, United States*

301. **Nsp8-TP25 Peptide as a Promising Therapeutic Agent Targeting the SARS-CoV-2 RdRp Complex**
Se-Mi Kim, PhD, *Center for Study of Emerging and Re-emerging Viruses, Korea Virus Research Institute, IBS, Republic of Korea*

302. **A potent small molecule inhibitor of Receptor Binding Domain and ACE2 receptor interaction**
Vidya Chitta Voina, M.S., *Department of Biotechnology and Bioinformatics, University of Hyderabad., Hyderabad, Telangana, India*

303. **Gingival vaccination as an antiviral vaccination strategy: Prospects for elderly vaccination**
Marni Cueno, D.Med.Sc., *Nihon University School of Dentistry, Tokyo, Japan*

304. **Potent Anti-COVID-19 Agent, GMS007, Made of Stable and Cell-Permeable Peptide Nucleic Acid (PNA)**
Hyunseok Hong, Ph.D., *Gifted MS, Incheon, Republic of Korea*

305. **Evaluation of Major Components of Green Plants, as Potential Therapeutics for SARS-CoV-2 variants**
Eun Ha Kim, *Institute for Basic Science (IBS), Daejeon, Republic of Korea*

306. **In Silico Identification and In Vitro Validation of Repurposed Compounds Targeting the RSV Polymerase**
Bo Liang, Ph.D., *Emory University School of Medicine, Atlanta, GA, United States*

307. **Targeting viral RNA as new approach for antivirals: from in silico to cells**
Gregory Mathez, M.S., *Institute of Microbiology, Lausanne University Hospital, University of Lausanne, Switzerland, Lausanne, Switzerland*

308. **Discovery of Nanosota-2, -3, and -4 as super potent and broad-spectrum therapeutic nanobody candidates against COVID-19**
Alise R. Mendoza, B.S., *Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN, United States*

309. **A New Cell Culture-based Assay IRINA to Assess Antiviral Susceptibility of Seasonal and Variant Influenza Viruses**
Vasiliy P. Mishin, Ph.D., *CDC, Atlanta, GA, United States*

310. **Intriguing Structure of the Influenza A Virus Genome - Are G-quadruplexes Potential Targets for Antivirals?**
Maria Nalewaj, M.S., *Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland*

312. **A natural compound and its synthetic derivative: towards new potent pan-coronavirus antiviral agents**
Karin Séron, PhD, *Center for Infection and Immunity of Lille, Univ. Lille, CNRS, Inserm, Institut Pasteur de Lille, Lille, France*

313. **A Favipiravir analogue and chain terminator, active against SARS-CoV-2**
Ashleigh Shannon, Ph.D, *AFMB, CNRS, Aix-Marseille University, Marseille, France*

315. **Imiquimod Inhibits the Multiplication of Coronaviruses Through the MAPK/ERK Pathway**
Josefina Vicente, Ph.D. Seeking, *Virology Laboratory, Biochemistry Department, University of Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina*

316. **Disruption of Spike Priming in Virus Entry: Tetrandrine's Potential Against Pan-coronaviruses**
Kun Wang, *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, China*

317. **Lycorine Inhibits Influenza Virus Replication Through Autophagy Pathways**
Haiyan Yan, *Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, China*

318. **Human guanylate-binding protein (GBP)1 inhibits replication of severe acute respiratory syndrome coronavirus type-2 through a mechanism distinct to GBP2 and GBP5**
Ruby Farrukee, PhD, *The University of Melbourne, Melbourne, Victoria, Australia*

319. **By interacting with cell junction and polarity proteins, the PDZ Binding Motif of SARS-CoV-2 Envelope protein constitute a major determinant of pathogenicity and appears as an innovative therapeutic target**
Flavio Alvarez, *Channel Receptors Unit, Neuroscience Department, Institut Pasteur, Paris, France*

320. **Identification of MARVAS110, a potent antiviral compound for Enterovirus D68 infection**
Yuhui Deborah Fong, *National University of Singapore,Singapore*

321. **Mechanism of Action of a 4'-Cyano Modified Nucleotide Analog Against a Platform of Diverse Polymerases of Respiratory RNA Viruses**
Calvin J. Gordon, Ph.D seeking, *Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada*

322. **Directed Evolution of SARS-CoV-2 Spike Protein Reveals Determinants of Fusion Route and Syncytia Formation**
Amal Rahmeh, PhD, *Universitat Pompeu Fabra, Barcelona, Spain*

323. **Novel SARS-CoV-2 entry inhibitors, 2-anilinoquinazolin-4(3H)-one derivatives, show potency as SARS-CoV-2 antivirals in a human ACE2 transgenic mouse model**
Sangeun Jeon, *Zoonotic Virus Laboratory, Institut Pasteur Korea, Seongnam-si, Republic of Korea*

324. **Evaluating SARS-CoV-2 Antiviral Activity Using the Drosophila Midgut Model**
Chaker El Kalamouni, Prof, *Université de La Réunion, UMR PIMIT, INSERM 1187, CNRS 9192, IRD 249, La Réunion, France*

325. **The immunobiotic Clostridium butyricum S45-5 displays broad spectrum of antiviral activity in vitro and in vivo**
Jong-soo Lee, *College of Veterinary Medicine, Chungnam National University, Daejeon, K*

326. **Anti-Respiratory Syncytial Virus activity of Sargassum fusiforme extract and its components in vitro and in vivo**
Jong-soo Lee, *College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea*

327. **Extracts of Aster tataricus and its component display broad spectrum of antiviral activity in Vitro and in Vivo by inducing antiviral state**
Jong-soo Lee, *College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea*

328. **Anti-Influenza effects of Lactobacillus reuteri BSA218 by Modulating Innate Immunity**
Jong-soo Lee, *College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea*

329. **Mesenchymal stem cells as immunomodulatory and regenerative agent for SARS-CoV-2 infection**
Erly Raras Savitri, *University of Bristol, Bristol, United Kingdom*

330. **Establishment of an integrated assay platform for supporting the discovery of antivirals and vaccines against human metapneumovirus infection**
Fusen Lin, *WuXi AppTec, Shanghai, China*

331. **Evaluation of the Antiviral Activity and Cytokine Response of EIDD-1931 and Ensitrelvir in a specialized 3D normal, human tracheal/bronchial (EpiAirway) tissue model infected with SARS-CoV-2**
Brett L. Hurst, Ph.D., *Utah State University, Logan, Utah, United States*

332. **Discovery and characterization of EGT710, an oral SARS-CoV-2 Mpro inhibitor and clinical candidate**
Stephanie Moquin, *Novartis, Emeryville, CA, United States*

333. **Comparative Analysis of Resistance Mutation Emergence in SARS-CoV-2 Under Single and Combination Antiviral Therapies**
Seong Cheol Min, *College of Medicine and Medical Research Institute, Chungbuk National University, Cheong ju-si, Chungchungbuk-do, Republic of Korea*

334. **From Barrier to Target: How Differentiation Impacts Upper Airway Epithelium Susceptibility to HMPV**
Patrice Guillon, Ph.D, *Institute for Glycomics, Griffith University, Gold Coast Campus, Qld, Australia, Southport, QLD, Australia*

335. **Intranasal Antivirals Against Respiratory Syncytial Virus: The Current Therapeutic Development Landscape**
Victor Baba Oti, *School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, Queensland, Australia*

350V. **Characterising mutational pressures exerted by nucleoside analogues on SARS-CoV-2 evolution**
I'ah Donovan-Banfield, M.S., *Department of Infection Biology and Microbiomes, University of Liverpool, Liverpool, United Kingdom*

351V. **NEPTUNO: A Phase III Clinical Study of Plitidepsin for the Treatment of Adult Patients with COVID-19 Requiring Oxygen Therapy**
Diego López-Mendoza, MD, PhD, *PharmaMar, Madrid, Spain*

352V. **A Comprehensive Depiction of Events and Signaling Pathways Involving in SARS-CoV-2 Spike Protein-mediated Syncytia Formation**
Xiaoben Pan, *Global Health Drug Discovery Institute, Beijing, China*

353V. **Predictive assessment of Imperata cylindrica antiviral properties against SARS CoV-2**
Foka Frank, Ph.D., *North West University, Mafikeng, North West Province, South Africa*

354V. **6-Azauridine Prodrugs as Anti-Influenza Inhibitors**
Omar Moukha-Chafiq, *Southern Research, Birmingham, Alabama, United States*

355V. **Exploiting DKA derivatives as SARS-CoV-2 nsp13 inhibitors active on viral replication**
Roberta Emmolo, PhD, *Dip. Scienze della Vita e dell'Ambiente, Università di Cagliari, Monserrato, Cagliari, Italy*

356V. **Travatrelvir, a Potent Inhibitor of SARS-CoV-2 Main Protease now in Phase 1 Clinical Trials, Shows a Superior Drug Resistance Profile in vitro Compared to Nirmatrelvir**
C David Pauza, PhD, *Traws Pharma, Inc., Rockville, Maryland, United States*

400V. **Identification of the new HSV-2 variant (HSV-2v) in two patients suffering from genital herpes not responding to valacyclovir therapy**
Graciela Andrei, PhD, *Rega Institute, KU Leuven, Leuven, Belgium*

401. **Helicase-primase inhibitor IM-250 efficiently controls herpes infections and recurrent disease by reducing the latent viral reservoir in animal models and shows sufficient exposure in a Phase 1 clinical trial**
Gerald Kleymann, Prof. Dr., *Innovative Molecules GmbH, Munich, Bavaria, Germany*

402. **HIV-1 Topoisomerase IIβ kinase as novel target to inhibit viral reverse transcription**
Anand Kumar Kondapi, PhD, *University of Hyderabad, Hyderabad, India*

403. **Discovery of Novel Aryl Triazolone Dihydropyridines (ATDPs) Targeting Highly Conserved Residue W229 as Promising HIV-1 NNRTIs**
Dongwei Kang, *Shandong Univresity, Jinan, Shandong, China*

404. **Discovery of novel phenylalanine derivatives as potent HIV capsid modulators with improved antiretroviral activity and metabolic stability**
Xiangyi Jiang, M.S., *Shandong University, Jinan, Shandong, China*

500. **Modeling the impact of antiviral therapy on liver disease associated with chronic hepacivirus infection**
Timothy P. Sheahan, Ph.D., *UNC Gillings School of Global Public Health, Chapel Hill, North Carolina, United States*

501. **Human Intestinal Enteroids as a new in vitro model for Hepatitis E Virus antiviral development**
Nanci Santos-Ferreira, *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

502. **Propiophenone Thiosemicarbazones as New Agents Against Bovine Viral Diarrhea Virus**
Matias Fabiani, *Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Ciudad de Buenos Aires, Buenos Aires, Argentina*

503. **Development and establishment of reporter replicons for antiviral drug discovery against human Noroviruses**
Myra De Torres Hosmillo, Ph.D, *University of Cambridge, Cambridge, Cambridgeshire, United Kingdom*

504. **Antiviral Inhibition of Human Norovirus ProPol Precursor**
Alice M. McSweeney, *Otago University, Dunedin, New Zealand*

505. **Exploring Antiviral Candidates for Human Norovirus: Utilizing the Pandemic Response Box's Open-Source Compound Repository**
Euan Docherty Webster, BSci (Hons), *University of Cambridge, Cambridge, Cambridgeshire, United Kingdom*

506. **PDZ Interactions as Determinants of Viral Pathogenicity and Therapeutic Targets**
Célia Caillet-Saguy, Ph.D, *Channel Receptors Unit, Neuroscience department, Institut Pasteur, Paris, France*

507. **Assay development to test viral intrinsically disordered regions as potential antiviral targets**
Vivienne Young, *University of Otago, Dunedin, New Zealand*

508. **Development of Novel Anti-HBV Agents: Characterization of the N-Hydroxypyridinediones (HPDs) as HBV RNase H Inhibitors**
Grigoris Zoidis, Ph.D., *National And Kapodistrian University Of Athens, Athens, Greece*

001. Virus Emergence at the Human-Animal Interface

Edward C. Holmes, FAA, FRS, The University of Sydney, Australia, Sydney, NSW, Australia

Viruses are diverse components of global ecosystems. Bulk RNA shotgun sequencing – metatranscriptomics – has transformed our understanding of the virosphere, providing a uniquely powerful means to describe the viral composition of any sample, and helping to reveal how viruses move across the human-animal interface and eventually emerge as new infectious diseases. Herein, I will show how metatranscriptomics, combined with advances in artificial intelligence (AI) technology that can integrate primary sequence and structural information to accurately and efficiently detect viral sequences, is providing new insights into fundamental aspects of virus evolution, ecology and emergence. I will identity the viruses that circulate at key components of the human-animal interface, including the wildlife trade, fur farms, live animal markets, and in urban environments in Australia, identifying those viruses which are likely to have the greatest potential for zoonotic emergence. I will also identify the fundamental drivers of virus diversity and evolution at the scale of individual ecosystems, revealing the impact of host barriers to cross-species virus transmission. Finally, I will show how a combination of metatranscriptomics and AI has led to the discovery of tens of thousands of novel RNA viruses, redefining our knowledge of the scale and composition of the virosphere.

002. Therapeutic Drug Development For Acute Viral Diseases: Lessons Learned and Future Perspective from a Clinician-Scientist and Can We Do Better?

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Acute viral infections continue to cause unexpected and explosive outbreaks with potentials for pandemics as emphatically demonstrated by the most recent COVID-19 pandemic. Besides vaccines, interventions such as effective antivirals play an important role in the armamentarium of public health tools to control outbreaks. However, development of therapeutics against acute viral infections has been challenging. In this talk, I will discuss lessons learned from the clinical development of therapeutics against a number of flaviviruses and SARS CoV-2 from bench to bedside. I will also share my thoughts on the value of therapeutics against acute viral diseases from the perspective of a practicing infectious diseases physician and researcher at a busy tertiary academic hospital.

003. My Battle Against Viruses

Professor Johan Hendrik Neyts, KU Leuven, Belgium

My laboratory www.antivirals.be develops antiviral strategies against neglected & emerging viruses including in the context of pandemic preparedness. I will present some selected stories. We developed, together with www.cd3.be an ultrapotent pan-serotype dengue virus inhibitor (JNJ-802) that blocks the interaction between two non-structural viral proteins of the virus. JNJ-802 is efficacious against dengue, as reported by J&J, in a human challenge model. We developed a class of highly potent pan-enterovirus inhibitors that target the 2C protein and that are highly effective in animal infection models. Together with Aligos Therapeutics, a potent coronavirus Mpro inhibitor (ALG-097558) is being developed that does not need, unlike nirmatrelvir, to be combined with ritonavir. We discovered a novel class of highly potent SARS-CoV2 inhibitors that target the viral Membrane protein and that thereby prevents viral assembly, an entirely novel druggable target of coronaviruses. An analogue in the series is more potent than nirmatrelvir in a mouse infection model. Annually ~60.000 people die of rabies, once neurological symptoms appear, mortality is 100%. We are developing physiologically relevant cell cultures models (including human brain organoids), established a high throughput antiviral screen against the rabies virus and are developing potent inhibitors that can be used to save the lives of those with symptoms. Human parainfluenza viruses (hPIV) can cause severe disease, in particular in the immunodeficient patient. We developed the first hPIV(3) infection model in mice and demonstrate that the parent nucleoside of remdesivir provides good protection in this model.

004. Viral Hemorrhagic Fevers: Challenges and Gains of Animal Model Research for Pre-Clinical Vaccine and Antiviral Screening

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Animal models and their use in translational research studies are critical to study pathological processes associated with viral infections and how drugs or treatments affect the entire organism, ideally resembling the same processes in humans. Applying these models first requires well-designed and controlled model development studies that consider both the experimental approach (challenge route, dose, viral species/strain) and animal species-specific characteristics (age, sex, anatomy, physiology, immunity). Our efforts focus on advancing antivirals and vaccine candidates for high-containment hemorrhagic fever viruses through pre-clinical screening in small animal models, like mice, hamsters, and guinea pigs. Given the importance of model knowledge, we also focus on advancing and developing novel animal models of viral hemorrhagic fever (VHF) diseases. Here, examples of pathogenesis and medical countermeasure screening studies for high-containment high-consequence viruses, like Ebola, Lassa, Crimean-Congo hemorrhagic fever and Nipah viruses, will be used to illustrate advances in model development. Approaches to clinical evaluation, monitoring, and endpoint criteria will be discussed, along with approaches for assessing virological and immunological indices. The value of advanced models and experimental methods for improving our understanding of disease and identifying promising candidates for preventing morbidity or improving clinical outcomes of VHF infections will also be presented. Finally, unique challenges and considerations for pre-clinical animal model studies conducted in high containment will be highlighted.

005. Oral Pharmacokinetics and Efficacy of Modified Oral Lipid RVn Prodrugs Against SARS-CoV-2 in Mice

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Karl Y. Hostetler, M.D., Department of Medicine, University of California at San Diego, United States

To develop potent orally bioavailable antivirals to treat RNA viruses of clinical concern we previously synthesized 1-O-octadecyl-2-O-benzyl-sn-glyceryl-P-RVn (ODBG-P-RVn, V2043), a lipid prodrug of GS-441524 monophosphate (RVn-P), and demonstrated its in vivo efficacy in a SARS-CoV-2 mouse model. Using structure/activity studies we identified two additional promoiety modifications, 3-fluoro-4-methoxy-benzyl (V2053) or 4-cyano-benzyl (V2067), that significantly increased in vitro antiviral activity compared to V2043 against many clinically important RNA viruses. We performed oral and IV pharmacokinetic (PK) studies and evaluated the in vivo efficacy of orally administered V2043, V2053 and V2067 compared to Molnupiravir, Remdesivir, and Obeldesivir (GS-5245) in prophylaxis and treatment models of murine SARS-CoV-2 B.1.351 infection. In both models, once daily oral V2043, V2053 and V2067 significantly reduced lung viral titers at d2 (~3 log10 reduction) and eliminated detectable virus at d4 post-infection while Obeldesivir and Remdesivir did not. Oral doses of up to 180 mg/kg of each phospholipid prodrug were well tolerated and Cmax levels of V2043, V2053 and V2067 at 60 mg/kg were 20.3, 7.7 and 24.9 micromoles/liter. Oral and IV single dose PK determined the % oral bioavailability of V2043, V2053 and V2067 to be 73.6%, 51.0%, and 57.0%. Our data shows that V2043, V2053, and V2067 are well tolerated, orally bioavailable and potent in vivo inhibitors of SARS-CoV-2. It is notable that on a molar basis V2043 and V2053 are at least 5 times more active than Obeldesivir while V2067 is 5 to 16.9 times more active in vivo than Obeldesivir.

006. Towards a Novel Host-Targeted Anti-Infective Strategy Against COVID-19 and Other Acute Respiratory Viral Diseases

Stephan Ludwig, PhD, Institute of Virology, Muenster, Germany
Andre Schreiber, Institute of Virology, Germany
Oliver Planz, University of Tuebingen, Tübingen, Germany

COVID-19 and other hyperinflammatory virus disease progresses in at least two stages, the first being dominated by damage caused by the virus itself, while the second stage occurs through a hyper-induction of cytokines. Previously, we have unraveled a strong dependence of IAV replication on the cellular Raf/MEK/ERK kinase pathway and also obtained evidence that the pathway acts immunomodulatory. Furthermore, we could show that also other viruses, such as RSV, BDV, or Hantaviruses are sensitive to MEK inhibition, suggesting that MEK inhibitors could act as novel broadly active antivirals with a dual benefit: directly, via impairing virus replication and indirectly, by re-balancing overshooting immune responses. Accordingly, we demonstrated that the MEK inhibitor Zapnometinib results in a sustained inhibition of SARS-CoV-2 propagation and also leads to reduced expression of virus-induced proinflammatory cytokines while the antiviral type I interferon response appears not to be affected. The compound also acts synergistic with direct acting antivirals (DAA) and moreover displays a high barrier towards emergence of resistance. The drug has successfully been proven to be save and well tolerated in humans in a phase I clinical trial (NCT04385420). Results of a phase II clinical trial employing Zapnometinib in hospitalized COVID-19 patients (RESPIRE, NCT04776044) indicate clinically relevant efficacy with a very favorable safety profile. Zapnometinib resulted in both, reduced virus titers and dampening of the cytokine response in patients. Thus, the concept of MEK inhibition as an anti-infective strategy is a clinically feasible and promising approach.

007. Exploring Viral RNA Methyltransferases for Antiviral Drug Design

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Our investigation deals with viral RNA methyltransferases, pivotal enzymes responsible for methylating the 5′ cap structure of viral RNA. Specifically, these methyltransferases target cap GTP at position 7 and the first nucleotide at position 2′. Our focus centers on methyltransferases from various viruses, with particular attention to two enzymes—nsp14 and nsp16/nsp10—from the SARS-CoV-2 virus. Our primary goal involves obtaining crystal structures of these enzymes to facilitate the design of inhibitors for crucial SARS-CoV-2 enzymes. Leveraging the structural model, we developed initial nanomolar inhibitors for the SARS-CoV-2 nsp14 N-7 methyltransferase, subsequently refining them through rational design. Recent successes include obtaining the crystal structure of an optimized inhibitor derivative in a complex with the nsp14 methyltransferase domain. Additionally, our team achieved a milestone by securing the first crystal structure of the nsp16/nsp10 complex with a non-selective pan-methyltransferase inhibitor. Further exploration revealed an allosteric cryptic cavity in nsp16/nsp10 capable of binding both covalent and non-covalent inhibitors. Shifting our focus to the monkeypox virus, we obtained the crystal structure of its 2′-O methyltransferase (VP39 protein), established an HTS assay, and screened a library of approximately 500 SAH derivatives. This effort led to the identification of several potent inhibitors for the VP39 methyltransferase, offering potential avenues for antiviral drug development.

008. Targeting Host Kinases to Identify Novel Broad-Spectrum Antiviral Strategies

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Marco Radi, University of Parma, Italy

A strategy to overcome the development of resistance to antivirals, and simultaneously to identify broad-spectrum molecules, is to target the host. In this context, finding an enzyme or a cellular protein, redundant for the cell but essential for the virus, is fundamental to limit cytotoxicity while maintaining antiviral activity. One of the possible targets is host kinases, druggable proteins largely studied for cancer but not yet exploited as antivirals. Thus, we studied inhibitors of Phosphatidylinositol 4-kinase IIIβ (PI4KIIIβ), which is required in the life cycle of different viruses, such as the formation of replication organelles. We identified bisthiazole derivatives active, as expected, on several members of Picornaviridae but retaining activity on members of the Flaviviridae and Coronaviridae. We are currently investigating the possibility to convert them into protacs to achieve a more potent inhibition. In parallel, we explored the efficacy of Src inhibitors, previously recognized for their activity against Flaviviridae, as potential ingredients for vaginal microbicides. Through testing various derivatives, we have identified compounds

showing activity against Zika virus, Monkeypox virus, HIV, and Herpes simplex virus type 2. Remarkably, the lead compound also exhibited effectiveness against the intracellular bacteria Chlamydia Trachomatis. Our ongoing research aims to assess the compounds activity against additional sexually transmitted pathogens, unravel the viral life cycle step inhibited, and subject them to testing in more relevant models.

009. UNAPP: A Unique Academic-Industrial Partnership for Antiviral Research in Pandemic Preparedness

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The ongoing COVID-19 pandemic highlights the critical need for preparedness against future outbreaks and pandemics. Lessons learned from this crisis emphasize the necessity of developing late preclinical or clinical-stage drug candidates with broad antiviral spectrum activity, akin to remdesivir and Paxlovid, which facilitate swift registration and clinical deployment in future pandemics. In response, the UTMB-Novartis Alliance for Pandemic Preparedness (UNAPP) has been established with NIH support, bringing together UTMB's prowess in infectious diseases and Novartis's expertise in drug discovery and development. Over the past two years, UNAPP has made substantial progress in antiviral drug research, while training for the next generation of drug hunters. This presentation provides an overview of UNAPP's unique program and its current progress, with a focus on creating essential biological tools for coronaviruses, flaviviruses, and Henipaviruses, as well as advancing our knowledge of the mode of action of Flavivirus NS4B inhibitor NITD-688, currently under Phase 2 clinical trials. We have revealed a unique antiviral mechanism of this inhibitor in blocking NS4B and NS3 interactions, which may pave a new avenue for developing next-generation Flavivirus NS4B inhibitors.

010. Molnupiravir Extends its Broader Spectrum of Activity to Human Norovirus and Rotavirus in 3D Human Intestinal Enteroids

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Winston Chiu, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium
Johan Hendrik Neyts, Professor, KU Leuven, Leuven, Belgium
Jelle Matthijnssens, KU Leuven, Rega Institute, Laboratory of Clinical and Epidemiological Virology, Belgium
Joana Rocha-Pereira, KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leiden, Belgium

Human norovirus (HuNoV) and human rotavirus (HRV) are the leading causes of gastrointestinal diarrhea. There are no approved antivirals and rotavirus vaccines are insufficient to cease HRV associated mortality. Likewise, treatment of chronically infected immunocompromised patients is limited to off-label compassionate use of repurposed antivirals with limited efficacy, highlighting the need for potent and specific antivirals. With the development of the human intestinal enteroids (HIEs), the replication of multiple circulating HuNoV and HRV genotypes can finally be studied and both in the same non-transformed and physiologically relevant model. Activity of previously described anti-norovirus or anti-rotavirus drugs, such as 2′-C-methylcytidine (2CMC), 7-deaza-2′-C-methyladenosine (7DMA), nitazoxanide, favipiravir and dasabuvir, was assessed against clinically relevant human genotypes using 3D-HIEs. 2CMC showed the best activity against HuNoV GII.4, while 7DMA was the most potent antiviral against HRV. Moreover, we evaluated the activity of molnupiravir, a broad spectrum antiviral used to treat Sars-CoV-2 infections. Molnupiravir and its active metabolite, N4-hydroxycytidine (NHC), inhibit HuNoV GII.4, HRV G1P[8], G2P[4] and G4P[6] in 3D-HIEs with high selectivity and show a potency comparable to 2CMC against HuNoV (EC50 ~ 0.5 μM). Moreover, molnupiravir and NHC block HRV viroplasm formation, but do not alter its size or subcellular localization. Taken together, molnupiravir inhibits both HuNoV and HRV replication, suggesting that the drug could be a candidate for the treatment of patients chronically infected with either one of these diarrhea causing viruses.

011. Targeting mpox Virus Resolvase (Mpr): In Vitro Assay Development and Inhibitors

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Poxviruses encode a resolvase to specifically cleave and resolve the four-way viral DNA Holliday junctions during genome replication. Repressing the resolvase function of vaccinia virus (VACV) inhibited the processing of viral DNA into unit-length genome and reduced VACV DNA replication. A reported inhibitor of fowlpox virus resolvase (Fpr) conferred antiviral potency against VACV in the low uM range, suggesting that pharmacologically inhibiting resolvase could provide a novel antiviral approach against mpox virus (MPXV). However, biochemical and structural studies on poxvirus resolvases have so far been limited to avipoxviruses, particularly fowlpox virus (FPV) and canarypox virus (CPV). Assays using mpox virus resolvase (Mpr) remain elusive, and Mpr inhibitors are unknown. We have developed and optimized a FRET-based Mpr assay using fluorescently labeled bulged DNA substrates known to be susceptible to resolvases. Using this assay, we have screened a library of curated in-house synthetic small molecules, and identified multiple hits with IC50 values in the nM to single-digit uM range. In the preliminary cell-based antiviral testing, some hits inhibited the VACV reporter virus by up to 100-fold at 10 uM. Our work reports the first Mpr inhibitors, and could open a new antiviral research area against MPXV.

012. Potential Genetic Determinants Affecting Virulence of Heartland Bandavirus Infection in Mice and Therapeutic Intervention with the Ribonucleoside Analog, EIDD-2749

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Heartland virus (HRTV) is an emerging tick-borne bandavirus that causes a febrile illness of varying severity in humans. The virus, transmitted by the Lone Star tick (Amblyomma americanum), was first isolated in 2009 from two patients in Missouri. More than 60 cases of HRTV disease have been reported, with four resulting in death. Fourteen states have reported HRTV infections; however, the tick vector spans much of the eastern and midwestern United States and wildlife serosurveys support the presence of the virus across this broad geographic range. No vaccines or approved therapies are available to prevent or treat HRTV disease. Here, we describe genetic changes arising during passage adaptation of the MO-4 strain of HRTV in AG129 mice, which markedly increased the virulence of the virus in mice at low challenge doses. We used this new infection model based on challenge with mouse-adapted HRTV to assess the therapeutic efficacy of the ribonucleoside analog, 4'-fluorouridine (EIDD-2749), and show that intervention starting after the onset of dramatic weight loss and other clinical signs of disease protects mice against lethal HRTV disease. Our findings provide insights into HRTV virulence in vivo and support further development of EIDD-2749 as a therapeutic intervention for severe cases of HRTV disease. Supported by the NIH (AI-152236, HHSN2722017000411 and 75N93019D00021) and the Defense Threat Reduction Agency (DTRA) contract MCDC2005-005).

013. Chasing an HIV Cure: The Intersection of Biological Sex and Latency Reversal

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The persistence of HIV-1 infection despite effective antiretroviral therapy (ART) is a major barrier to an HIV cure. While women constitute one half of people living with HIV disease, they are under-represented in HIV cure studies. Sex-based differences may influence the outcome of HIV infection and similarly the HIV reservoir. Variations in estrogen concentration have been proposed to explain sex-differences observed in HIV infection, clinical progression and reservoir size. Estrogen receptor alpha (ERα) is reported to directly influence HIV-latency and transcription. However, unlike our knowledge of ERα regulation in breast cancer cells, the regulation of ERα in immune cells is less well-defined. Furthermore, in the latency reversal and clearance HIV cure strategy, the influence of latency reversal agents (LRAs) on ERα is unknown. Our recent study exploring HIV persistence in women, including efforts to obtain a better understanding of ERα regulation in CD4+ T cells will be highlighted. These include, evaluation of nuclear localization of ERα and responsiveness to estrogen receptor modulators and degraders, and assessment of the impact of different LRA classes (HDAC and BET inhibitors, PKC and STING agonists, SMAC mimetics) on ERα mRNA and protein. Lastly, new approaches to latency reversal and clearance, singly and in combination, are being developed and will also be discussed as these new approaches must also be broadly assessed in different populations, including women.

014. Drug Discovery Efforts Towards Human Metapneumovirus

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Human metapneumoviruses (HMPVs) have emerged in the past decades as an important global pathogen that causes severe upper and lower respiratory tract infections. Children under the age of 2, the elderly and immunocompromised individuals are more susceptible to HMPV infection due to their suboptimal immune system. Reports have rapidly described its epidemiology, biology, and pathogenesis, but a successful vaccine therapy as well as an effective drug candidate are missing to date. HMPV fusion (F) protein is a key viral surface glycoprotein that is essential for target-cell recognition, attachment, and entry. Heparan sulfate proteoglycans extensively decorate the human cell surface and were previously described as an important cellular receptor for HMPV F, underlining the role of glycans as viral attachment factors.

In an effort to contribute towards an antiviral for HMPV, two strategies were followed. Firstly, drug repurposing of an FDA-approved drug library in an in vitro medium-throughput screening was conducted. Secondly, a multidisciplinary approach was utilised to understand how human metapneumovirus fusion protein binds to its glycan receptors on the host cells. Rapid discoveries of novel hit candidates provide promising novel templates for the drug design to prevent or treat HMPV infection.

015. Identification of Novel Small-Molecule Inhibitors of SARS-CoV-2 by Chemical Genetics

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There are only five approved small molecule antiviral drugs for treating COVID-19. Among them, three are nucleotide analogues (remdesivir, JT001, and Mmlnupiravir), while the other two are protease inhibitors (nirmatrelvir and ensitrelvir). Antiviral resistance, unfavourable drug-drug interaction and toxicity have been reported in previous studies. Thus there is a dearth of new treatment options for SARS-CoV-2. In this work, a three-tier cell-based screening was employed to identify novel compounds with anti-SARS-CoV-2 activity. One compound, designated 172, demonstrated broad-spectrum antiviral activity against multiple human pathogenic coronaviruses and different SARS-CoV-2 variants of concern. Mechanistic studies validated by reverse genetics showed that compound 172 inhibits the 3-chymotrypsin-like protease (3CLpro) by binding to an allosteric site and reduces 3CLpro dimerization. A drug synergistic checkerboard assay demonstrated that compound 172 can achieve drug synergy with nirmatrelvir in vitro. In vivo studies confirmed the antiviral activity of compound 172 in both Golden Syrian Hamsters and K18 humanized ACE2 mice. Overall, this study identified an alternative druggable site on the SARS-CoV-2 3CLpro, proposed a potential combination therapy with nirmatrelvir to reduce the risk of antiviral resistance and shed light on the development of allosteric protease inhibitors for treating a range of coronavirus diseases.

016. Activating the RIG-I Pathway by RNA Agonists or Small Molecule Drugs Triggers Innate Immune Programming to Control Infection by Influenza A virus and SARS-COV2

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RNA viruses continually evolve during the course of transmission and infection, such that viral genetic variation facilitates viral evasion of host natural and vaccine immunity, and confers resistance to antiviral therapy. Influenza A virus (IAV) and SARS-COV2 each mediate ongoing endemic infections and pose new pandemic threats. Host innate immune responses are rapidly deployed within minutes to hours post IAV or SARS-COV2 exposure to establish innate antiviral states that serve to suppress virus replication and spread while coordinating and polarizing the adaptive immune response. Viral genome sensing by retinoic acid inducible gene-I (RIG-I) can lead to innate immune stimulation to trigger the activation of key transcription factors such as IRF3 and NFKB to drive the production of antiviral effector genes, which includes cytokines such as interferons (IFNs) and interleukins, and a wide range of IFN-stimulated genes (ISGs). We have developed a RIG-I binding and activating RNA (RAR) that can be reliably delivered in vitro and in vivo using novel RNA delivery technologies such as Lipid InOrganic Nanoparticle (LION). In addition, we have developed small molecule therapeutics (SMT)s that bind RIG-I or modulate key components of the RIG-I pathway. We assessed the antiviral activity of each in prophylactic and therapeutic model applications against IAV and SARS-COV2. RAR and SMTs induce an innate immune response that can control both IAV and SARS-COV2 infection in vitro and in mouse models. Our work shows that targeting the RIGI pathway is an attractive and effective host-directed intervention, which can be used prophylactically or therapeutically against viral infections.

017. Understanding and Inhibiting SARS-CoV-2 NiRAN Domain Catalytic Activities Through Structural Studies and Large-Scale Docking

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The enzymatic activity of the NiRAN domain, encoded in SARS-CoV-2 nsp12, is essential for viral replication in coronaviruses and possesses three distinct activities associated with modification of the nsp9 N-terminus. These activities include NMPylation, RNAylation, and mRNA capping. While RNAylation and mRNA capping have roles in the production of the 5′ mRNA cap, the role of NMPylation in viral replication remains unclear. We determined high-resolution cryo-electron microscopy structures visualizing NMPylation and mRNA capping catalytic intermediates in complex with their preferred substrates. These structures reveal the determinants of substrate recognition and coordination of catalytic Mg2+ ions in the active site, providing insight into the enzyme's multiple catalytic mechanisms. We then used in silico docking with ultra-large libraries containing millions of diverse, readily synthesizable molecules. Candidate molecules were selected for synthesis after filtering the top scoring molecules for novelty and desired interactions with the NiRAN active site. These molecules were tested in vitro for their ability to bind to the NiRAN domain and inhibit mRNA capping. In summary, we report on the structural basis of the enzyme's plasticity in substrate recognition and catalytic activity, and how we leverage these structures to establish a pipeline to identify novel chemotypes that bind the NiRAN domain to further the developments of antivirals against coronaviruses.

018. Combating Emerging Henipaviruses

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Henipaviruses (HNVs) are emerging, highly-pathogenic viruses without approved human vaccines or therapies. The prototypical HNVs, Nipah virus (NiV) and Hendra virus (HeV), are responsible for annual outbreaks in India and Bangladesh, and instances of spillover to humans have been documented in several other countries, including Australia, China, Malaysia, Singapore, and the Philippines. These viruses enter host cells through a two-step process: first, the receptor binding protein (RBP) mediates cell attachment, and then the fusion (F) glycoprotein mediates the fusion of the viral and host cell membranes. As a result, the RBP and F proteins are the primary targets for vaccine and therapeutic development against HNVs.

In August 2022, a novel HNV named Langya virus (LayV) was isolated from patients with severe pneumonic disease in China. This virus is closely related to Mòjiāng virus (MojV), and both are divergent from the bat-borne HNV members, NiV and HeV. The spillover of LayV is the first instance of a HNV zoonosis to humans outside of NiV and HeV, highlighting the continuing threat this genus poses to human health. To better understand these new viral threats we made use of cryogenic electron microscopy (cryoEM) to determine the structures of both F and RBP LayV glycoproteins. Using phage display, we identified human antibodies that target LayV and MojV and determined the structure of a novel epitope within LayV RBP. Our work carries implications into vaccine and therapeutic development for HNVs, with the overall aim of improved pandemic preparedness against these emerging viruses.

019. Design of SARS-CoV-2 Papain-like Protease Inhibitor with Antiviral Efficacy in a Mouse Model

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The emergence of SARS-CoV-2 variants and drug-resistant mutants calls for additional oral antivirals. The SARS-CoV-2 papain-like protease (PLpro) is a promising but challenging drug target. PLpro cleaves viral polyproteins and is also involved in antagonizing host immune response. In this study, we discovered a new drug binding site, Val70Ub, and subsequently designed and synthesized a library of noncovalent PLpro inhibitors that bind to Val70Ub site and the known BL2 groove pocket. Potent compounds inhibited PLpro with inhibitory constant Ki values from 13.2 to 88.2 nM. The co-crystal structures of PLpro with eight leads revealed their interaction modes. The in vivo lead Jun12682 inhibited SARS-CoV-2 and its variants, including nirmatrelvir-resistant strains with EC50 from 0.44 to 2.02 μM. Oral treatment with Jun12682 significantly improved survival and reduced lung viral loads and lesions in a SARS-CoV-2 infection mouse model, suggesting PLpro inhibitors are promising oral SARS-CoV-2 antiviral candidates. Collectively, our study revealed Jun12682 as the first drug-like PLpro inhibitor with in vivo antiviral efficacy in a SARS-CoV-2 infection mouse model, further validating PLpro as a viable antiviral drug target. The preprint of our study can be found: https://www.biorxiv.org/content/10.1101/2023.12.01.569653v1

020. Identification of Adenosine Analogues that Inhibit the N7 Methyltransferase Activity of SARS-CoV-2

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SARS-CoV-2 is the etiological agent of Covid-19. Coronaviruses (CoVs) are enveloped viruses responsible for various pulmonary infections. SARS-CoV-1, SARS-CoV-2 and MERS-CoV induce severe pneumonia.
CoVs have a single-stranded genomic RNA with positive polarity, encoding 20 proteins, 16 of which are conserved non-structural proteins (nsp). Among these 16 nsp, the nsp14 and nsp16 proteins have a methyltransferase activity involved in cap structure synthesis. After transfer of the cap to the 5′ end of viral RNA (GpppA2′-OH-RNA), the nsp14 enzyme methylates the N7 position of guanine (G) from S-adenosylmethionine (SAM), forming cap-0 (7MeGpppA2′-OH-RNA). Then, a second methyl group is transferred to the first nucleotide's ribose in the 2′OH position by the nsp10/16 complex, forming cap-1 (7MeGpppA2′OMe-RNA). These methylations stabilize RNAs against degradation and promote translation initiation, while limiting detection of viral RNA by innate immunity sensors. The COVID-19 pandemic highlights the need to develop new therapies targeting the SARS-CoV-2 replicative machinery. Here, we have developed a series of bi-substrate inhibitors mimicking SAM and the viral RNA cap. We show that some compounds specifically inhibit the N7-MTase activity of nsp14 with subnanomolar IC50, without inhibiting other viral MTases as well as human N7-MTase. Docking experiments reveal that the specificity of inhibition could result from the protrusion of their phenyl or 3-quinoline group in the SAM entry channel of nsp14 MTase, which is absent in hN7-MTase. This demonstrates the possibility of synthesizing highly specific inhibitors based on adenosine nucleoside analogues.

021. Nanobodies Against COVID-19 and Other Emerging Viruses

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The COVID-19 pandemic highlighted the limitations of conventional antibodies as antiviral therapeutics, such as their expensive cost, limited effectiveness, inability to combat emerging virus strains, and reliance on injections for administration. Nanobodies, which are single-domain antibodies, offer potential as antiviral therapeutics. At the Midwest Antiviral Drug Discovery (AViDD) center, one of our aims is to develop nanobodies into powerful, broad-spectrum, safe, affordable, and user-friendly antiviral treatments. We have identified several anti-SARS-CoV-2 nanobodies, termed Nanosotas, through camelid phage display libraries and from vaccinated alpacas. Among these, Nanosota-2 shows exceptional potency against the original SARS-CoV-2 strain, Nanosota-3 is highly effective against the Omicron variant, and Nanosota-4 works against both SARS-CoV-1 and SARS-CoV-2. These nanobodies stand out for their high potency and broad antiviral range, are cost-effective, can be quickly modified to tackle new viral variants via phage display, and have the potential to be administered via inhalers. We have also created highly potent nanobodies against the Ebola virus, named Nanosota-EBs. The Nanosota series represent promising therapeutic candidates for dealing with current viruses and preparing for potential future viral pandemics.

022. Intranasal administration of a live attenuated vaccine derived from NSP16-deficient SARS-CoV-2 confers sterilizing immunity in rodent models

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We present a potential solution to the lack of mucosal and sterilizing immunity provided by current mRNA, adenoviral vectored, and inactivated vaccines against SARS-CoV-2. Our candidate live attenuated vaccine, named d16, is derived from a strain of SARS-CoV-2 in which the NSP16 gene encoding 2′-O-methyltransferase is disrupted by a point mutation. In tests on hamsters and transgenic mice, d16 was found to be non-pathogenic and asymptomatic, yet robustly stimulated humoral and cell-mediated immune responses. A single intranasal dose of d16 resulted in sterilizing immunity in both the upper and lower respiratory tracts of hamsters, preventing viral spread in a contact-based transmission model. The neutralizing antibodies elicited by d16 also cross-reacted with several SARS-CoV-2 variants. Our work demonstrates the potential of NSP16-deficient SARS-CoV-2 as a basis for developing live attenuated vaccines. Further preclinical studies of d16 could lead to improvements in safety, transmissibility, immunogenicity, and efficacy.

023. Development of Broad-Spectrum Antiviral Drugs

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In the past twenty years, viral diseases had a significant impact on the human society. By constructing a multidimensional, multi-target, structurally diverse library of antiviral compounds, some drug candidates were obtained. At the onset of the COVID-19, based on the structure of SARS-CoV-2 Mpro, FB2001 was designed and synthesized, which exhibited broad-spectrum, potent antiviral activity. FB2001 does not require co-administration of ritonavir and shows potent activity against various clinically mutant strains. Injectable and nebulized forms of FB2001 are undergoing international multicenter Phase II/III trials, with related work published in Science as cover paper. [1,2] Additionally, some inhibitors also were designed to target various stages of the coronavirus lifecycle. For example, a drug candidate DC406068 was designed to inhibit Mpro and CTSL, which exhibits broad-spectrum antiviral activity against Coronaviruses and Ebola virus. DC403113 can inhibit TMPRSS2, CTSL, and Calpain, which shows effective broad-spectrum anti-coronavirus activity in vitro and in vivo. Based on the 3C/3CLpro, DC402209 and DC402267 were obtained which exhibit potent activity against enteroviruses, noroviruses and coronaviruses. And compounds A43 and DC056204 exhibited potent inhibitory activity against N protein. Based on the structure of CCR5, the HIV drug candidate Thioraviroc was designed and synthesized, which can be used in combination therapy. In clinical trials, it has shown good safety, tolerability, and significantly reduces viral load while increasing CD4 cell count.

[1] Hong Liu et al; Antivir. Res., 2022, 208, 105450
[2] Hong Liu et al; Science, 2020, 368: 1331-1335.

024. Some capsid/core assembly modulators (CAMs) can induce an inhibition of HBV RNA biogenesis

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Amongst newly investigated anti-HBV drugs are Capsid Assembly Modulators (CAMs), which primarily target the assembly of the core/HBc protein into nucleocapsid, and thus prevent neo-synthesis of new HBV genomes, as nucleos(i)de analogues do. But in addition to this primary mode of action, CAMs can also prevent cccDNA establishment into newly infected cells and impair HBeAg biogenesis, when used at higher concentrations. Moreover, our unpublished in vitro and in vivo results led us to hypothesize that CAMs could have an effect on HBV RNA biogenesis.

Here, we studied, using HBV-infected dHepaRG or primary human hepatocytes and standard molecular virology techniques, the effect of several CAMs (types E and A) on HBV RNA biogenesis.

Using two potent prototypic CAMs, we have shown that only the CAM-A was capable to significantly decrease the level of intracellular HBV RNAs, and subsequently the level of secreted HBsAg. The effect was stronger in dHepaRG cells, as compared to PHH. Run-On experiments and RNA decay assays allowed us to show that CAM-A was targeting cccDNA transcription. Long-term treatment with CAM-A was associated with a loss of capacity to co-immunoprecipitate cccDNA with anti-HBc antibody, suggesting either a "purge" of HBc from cccDNA or a misfolding. This CAM-A-induced inhibition of cccDNA transcription was associated/correlated with a reduction of the expression of HNF4a, an hepato-specific transcription factor that plays a major role in cccDNA transcription.

In this work we described another possible mode of action of some CAMs, which would consist in an inhibition of cccDNA transcription, resulting from a possible inhibition of HBc dimer functions.

029. Progress Towards Hepatitis B Cure

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Chronic hepatitis B virus (HBV) infection (CHB) affects approximately 300 million people worldwide, resulting in liver disease, including liver cancer. Although there is an effective preventative vaccine and suppressive antiviral therapies, there is no cure and almost 1 million deaths annually are directly attributed to HBV infection. There is global consensus that curing HBV will likely require approaches that target multiple aspects of the HBV replication cycle and stimulate antiviral host immune responses. The advent of direct acting antiviral cures for hepatitis C virus has raised expectations for HBV cure, however the highly complex nature of the HBV replication cycle provides considerable challenges not faced by the HCV cure field. Professor Revill will explore current advances towards HBV cure and discuss the challenges that need to be overcome to achieve this important goal.

030. Beyond Retroviruses: Restriction of Flavivirus Replication by TRIM5α

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TRIpartite Motif (TRIM) proteins belong to a large protein family, many of which are inducible by type I interferon and serve to suppress virus infection through direct interactions with viral proteins. Primate TRIM5α is a consequential inhibitor that suppresses lentivirus replication (e.g HIV-1) in a highly host species- and virus species-specific fashion to limit cross-species transmission of these viruses. Importantly, the antiviral effects of TRIM5α have been thought to function exclusively in the context of lentivirus

infection. Our research interests center on the flaviviruses that include significant pathogens that have emerged into human populations from primates (e.g. dengue virus, Zika virus, yellow fever virus) prompting us to determine whether TRIM5α could also function to inhibit flavivirus replication. Surprisingly, this work has revealed a new function for TRIM5α as a potent restriction factor for replication of specific flaviviruses. The mechanisms of inhibition, flavivirus escape, and the implications for therapeutic targeting will be discussed.

031. Liver-targeted therapeutic siRNAs against highly conserved yellow fever virus genomic sequences effectively limit infection and mortality in a hamster model

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Despite the availability of an effective vaccine, yellow fever virus (YFV) remains a significant cause of morbidity and mortality in endemic regions. Infection with YFV may result in extensive hepatocellular injury, steatosis, and necrosis. There is an urgent need, therefore, for effective hepatoprotective antivirals. Liver-targeted therapeutic siRNAs offer a novel approach to limit viral replication and hepatocellular damage by silencing highly conserved regions of the YFV genome. Here, a panel of siRNAs directed against conserved YFV genomic regions was initially screened in cell culture to define the intrinsic antiviral activity of each individual sequence. In a hamster model of severe yellow fever (YF), an optimized pan-genotypic liver-targeted siRNA, administered as a single subcutaneous injection 24 hours post-infection (p.i.), effectively limited infection, reducing serum viral load by ~3 log10 on day 4 p.i. At 6 days p.i., liver viral load was reduced by 2 log10 and serum ALT levels were reduced by 50%, consistent with limitation of hepatocellular injury. While vehicle treated controls exhibited significant weight loss and only 30% survival, weight loss was limited in animals receiving pan-genotypic siRNA treatment and no animal deaths occurred. Furthermore, additional studies evaluating liver-targeted siRNAs for YFV prophylaxis similarly demonstrated robust antiviral and hepatoprotective activity lasting for one-month post-treatment. Liver-targeted pan-genotypic siRNAs directed against highly conserved viral genomic sequences offer a novel therapeutic approach for the treatment of YF and prevention of life-threatening severe liver disease.

032. Ingestion of the antiviral drug JNJ-A07 by mosquitoes during blood-feeding significantly reduced dengue virus transmission

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Dengue virus (DENV) is the most widespread mosquito-borne virus worldwide, but no antiviral therapies are available yet. The pan-serotype DENV inhibitor JNJ-A07 has shown potent activity in a mouse model (PMID: 34616043). It remains unknown

whether an antiviral drug ingested by mosquitoes could inhibit virus replication, and thus reduce transmission to other hosts. Here, we investigated the antiviral activity of JNJ-A07 when administered in the bloodmeal to Aedes aegypti mosquitoes. Exposure to JNJ-A07 had no detrimental effect on mosquito lifespan (100 µM) or fecundity (25 µM). When mosquitoes fed on a DENV-2 infectious bloodmeal spiked with JNJ-A07 (2 µM, based on in vivo PK data), DENV infection and dissemination in mosquitoes was prevented. Pre-exposure prophylaxis with JNJ-A07 (2 µM) given six days prior to a DENV-infectious bloodmeal reduced the infection rate (IR, number of infected mosquito bodies) to 0% at day 7 post-infection (vs control: 64%). Interestingly, JNJ-A07 exposure (2 µM) to mosquitoes already infected with DENV disrupted the ongoing infection, decreasing virus dissemination to secondary organs such as the salivary glands (18.2% vs control: 95%), resulting in reduced transmission. Moreover, LC-MS/MS analysis showed that JNJ-A07 remained within the mosquito body at levels above the in vitro EC90 for seven days post-bloodmeal. Our findings highlight the potent anti-DENV activity of JNJ-A07 when ingested by Aedes aegypti mosquitoes via blood-feeding. This antiviral effect persisted under multiple conditions. Modelling on the empirical data is currently ongoing to estimate the impact of mosquito exposure to JNJ-A07 on the magnitude of a DENV outbreak.

033. Structural basis of Dengue and Zika virus NS1 multimerization and antibody recognition

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The genus of flavivirus includes many mosquito-borne pathogens, such as Zika (ZIKV) and the four serotypes of dengue (DENV1-4) viruses. Billions of people are affected as evidenced by epidemics and endemicity in many countries with no antiviral therapeutics available. Severe dengue and Zika infections are characterized by endothelial dysfunction associated with the secreted nonstructural protein 1 (sNS1), making it an attractive vaccine antigen and biotherapeutic target. However, the biologically relevant structure of sNS1 remains an enigma. sNS1 is believed to be secreted as hexamers that could dissociate and bind to epithelial cell membrane based on recombinant sNS1 (rsNS1) samples. We found that infection-derived sNS1 (isNS1) appeared as a ~250 kDa complex of NS1 and ApoA1 and determined the cryoEM structures of isNS1 and its complex with a monoclonal antibody. The major species of isNS1 is a complex of the NS1 dimer partially embedded in a High-Density Lipoprotein (HDL) particle. Cross-linking mass spectrometry (XL-MS) studies confirmed that the isNS1 interacts with the major HDL component ApoA1 via the NS1 wing and hydrophobic domains. A similar isNS1-HDL complex is also observed in sera from DENV-infected mice, ZIKV-infected mice, and a human dengue patient. We further present high resolution cryoEM structures of ZIKV rsNS1 alone and in complex with human anti-NS1 antibodies. We observed asymmetric tetramers of ZIKV rsNS1 that have the propensity to form filaments and uncovered a new subset of antibody-NS1 complex. Overall, our findings provided new insights into NS1 multimerization and the mechanistic basis of the protection conferred by antibodies targeting NS1.

034. Identification of mRNA processing machinery as druggable host factor targets for dengue virus infection

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Arboviruses, including endemic viruses such as dengue virus (DENV) and re-emerging ones such as Zika virus (ZIKV), are the etiological agents of many recent outbreaks, and threaten more than half of the world's population. There are currently no effective antiviral therapeutics or vaccines against many of these viruses. While antiviral drug development has traditionally focused on 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 of drug panels. We have taken a more directed approach to identify host factors and pathways that are targeted by DENV during infection. Using a mass spectrometry-based proteomic approach, we first generated a comprehensive DENV-human protein interactome in the context of DENV infection. We found that the mRNA processing pathway was a significant target during DENV infection, as host proteins involved in multiple steps of the pathway were found to interact with multiple DENV proteins. We further characterized the interaction between the DENV non-structural 3 (NS3) protein and the mRNA processing pathway, and revealed the functional significance of these interactions to DENV replication by targeting the pathway using RNA interference and small molecules.

035. Discovery of pan-flavivirus protease inhibitors

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Our research targets proteases from pathogenic flavi-, alpha- and coronaviruses and aims to develop broad-spectrum peptide-based inhibitors. Peptides may be derived from the substrate recognition sequence or identified de novo. We have a particular interest in peptide modifications that can (i) enhance metabolic stability by greater resistance towards proteolysis, (ii) promote biological uptake across cell membranes, (iii) allow for covalent interaction, and (iv) decrease the entropic penalty of binding by locking the peptide in the active conformation. After initial studies with covalently binding peptides of limited stability and selectivity, we explored constrained peptides. We developed different peptide cyclisation and stapling strategies using biocompatible chemistry. Using these approaches in small screening campaigns, we were able to identify various active cyclic and bicyclic peptides with improved proteolytic and plasma stability. In addition, we discovered macrocyclic peptides with novel binding modes using mRNA display screenings. We further characterised recombinant proteases from ten flaviviruses and conducted a comprehensive cleavage site analysis. We developed two bicyclic peptide inhibitors that displayed nanomolar inhibition across all ten proteases.

036. Combination therapy of approved drugs potentiates broad-spectrum antiviral activity against alphaviruses in human skin fibroblasts and mice

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Alphaviruses such as chikungunya virus (CHIKV) pose a significant threat to global public health, but no specific antiviral therapies are currently available. Here, we evaluated combinations of drugs that are already approved for other viral infections (sofosbuvir (SOF), molnupiravir (MPV) and favipiravir (FAV)) against CHIKV, Semliki Forest virus (SFV) and Sindbis virus (SINV). The drug combinations MPV + SOF and SOF + FAV resulted in antiviral synergy against CHIKV in human skin fibroblasts with Bliss scores of 12 and 21, respectively. SOF + FAV also showed synergy against SFV, whereas FAV + MPV conferred synergistic activity against SINV in Vero cells, demonstrating the enhanced potency of drug combinations against multiple alphaviruses in vitro.

In a mouse model of CHIKV arthritis, high monotherapy doses of MPV or FAV significantly reduced footpad swelling and infectious virus titers in serum, joints, and liver at day 3 post infection (pi), while SOF did not have a significant effect. With suboptimal monotherapy doses of MPV (10 mg/kg) or SOF (80 mg/kg), significant footpad swelling was observed. In contrast, footpad swelling was absent when the two drugs were combined. Moreover, MPV + SOF decreased the infectious virus titers in serum with 2 log10 TCID50/mL, compared to 0.1 or 1 log10 by SOF or MPV as a monotherapy. Thus, combining MPV and SOF resulted in enhanced anti-CHIKV activity in mice. In vivo studies with other drug combinations are ongoing.

Collectively, these in vitro and in vivo data demonstrate the enhanced antiviral potency of oral drug combinations for alphavirus infections.

037. Accelerating Antiviral Discovery with Artificial Intelligence

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The discovery of small molecule therapeutics is a critical part of pandemic response and preparedness. For pandemic response, a key challenge is discovering promising candidates in time to impact an evolving pandemic; for pandemic preparedness, the challenge is systematically finding broad-spectrum chemical starting point against diverse classes viral proteins to provide robust starting points. In my talk, I will discuss how Machine Learning can enable rapid drug discovery, as well as drugging previously untapped viral targets. A key theme will be the interplay between machine learning and structural biology. I will illustrate our approach with our recent work on SARS-CoV-2 Mpro, PLpro, and macrodomain, as well as work on pandemic preparedness directed at enteroviruses and flaviviruses.

038. Atomistic Model of the Coronavirus nsp3/nsp4 Double Membrane Vesicle Pore

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Coronavirus cellular infection results in significant membrane remodeling, leading to the formation of double membrane vesicles (DMVs). These vesicles are thought to provide a protective environment for viral replication to occur. Prior work has determined that DMVs have characteristic pores formed from the ORF1ab proteins nsp3 and nsp4, which facilitate export of newly synthesized viral RNA. Cryo-electron tomography analysis has revealed that the pores have six-fold symmetry, with a distinctive cytosolic crown-like structure. Using Alphafold and other methods, we report the first complete atomistic models of a DMV pore for both murine hepatitis virus (MHV) and SARS-CoV-2, which fit the available cryo-ET maps. We find that six subunits of nsp3 span the cytosolic crown, outer membrane, and DMV gap. This hexamer is surrounded by a dodecameric ring of nsp4 which spans the outer membrane, the DMV gap, and the inner membrane, completing the channel. Importantly, we find that this pore can be formed prior to nsp5 protease cleavage of the polyprotein, and formation of the pore itself appears to activate the protease. With the nsp4 ring forming from six subunits derived from pp1a (nsp4-nsp10) and six subunits derived from pp1ab (nsp4-nsp16), the stoichiometry of the cleaved products is consistent with our previous proposed model of a 60+ subunit hexameric replication complex. The model provides a clear mechanism for trapping the proteins within the DMV and has wide ranging implications with respect to membrane remodeling and DMV formation, polyprotein processing, the location of viral RNA replication and transcription, and the mechanism of action of several classes of inhibitors.

039. Antiviral therapy optimization for SARS-CoV2: a mathematical modeling approach

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Several antiviral drugs demonstrated efficacy against SARS-CoV-2 infection by lowering viral load and preventing hospitalization and death. Nirmatrelvir/ritonavir reduced hospitalization and death by 89% in a randomized clinical trial. However, it has been associated with viral rebound in real-world settings and failed as post-exposure prophylaxis. Molnupiravir reduced viral load and decreased hospitalization and death, but less than nirmatrelvir/ritonavir. Remdesivir substantially reduced hospitalization despite no difference in viral load reduction 7 days after therapy. We calibrated mechanistic mathematical models of viral dynamics combined with each drug's pharmacokinetic and pharmacodynamic models to

recapitulate the viral dynamics observed in several trials assessing these agents. Our model produced instances of viral rebound in individuals treated with nirmatrelvir/ritonavir, identified early treatment as a key risk factor for rebound, and predicted that early therapy can suppress innate immune responses and preserve susceptible cells without eliminating infected cells, allowing viral rebound after 5 days of treatment. We showed that extending treatment durations to 10-15 days limits the likelihood of viral rebound. For molnupiravir, our model accounts for defective viruses created by molnupiravir and shows how it leads to underestimation of the drug effect in the trial. Finally, our model demonstrates that remdesivir lowers viral load in the 3 days after therapy to achieve clinical benefit, despite equal viral loads with placebo on day 7. Our modeling approach provides a comprehensive framework to accurately predict clinical trial results for SARS-CoV-2 treatments

040. Click Chemistry-based Rapid Identification and Crystallographic Studies of Novel 1,2,3-Triazole-bearing Diazabicyclooctane Derivatives as Non-Covalent SARS-CoV-2 Mpro Inhibitors with Potent Antiviral Activity and Improved Drug-resistance Profile

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The threat of COVID-19 persists, and it remains imperative for medicinal chemists to develop broad-spectrum antiviral drugs that exhibit improved drug-resistance profiles while minimizing adverse reactions. The main protease (Mpro) is a promising target for anti-SARS-CoV-2 drug design. In this study, to rapidly discover potent Mpro inhibitors, a focused library containing 268 compounds was established on 96-well plates utilizing click chemistry. Through in-situ screening, novel 1,2,3-triazole derivatives were identified as highly potent Mpro inhibitors with significant anti-SARS-CoV-2 activity. Among them, both C5N17 (IC50 = 0.18 μM) and C5N21 (IC50 = 0.15 μM) exhibited prominent enzyme inhibitory activity. Enantiomer C5N17B (IC50 = 0.12 μM, EC50 = 0.08 μM) exhibited significant enzyme-inhibitory potency and excellent antiviral activity in Calu-3 cells. All these compounds exhibited low cytotoxicity (CC50 > 100 μM). Significantly, C5N17B showed higher potency than the approved drug nirmatrelvir (IC50 = 91 nM, EC50 = 1.5 μM) and reached the same level as ensitrelvir (EC50 < 0.1 μM). Notably, C5N17B displayed potent antiviral activities against various SARS-CoV-2 variants (EC50 = 0.13 – 0.26 μM) as well as HCoV-OC43 and HCoV-229E, indicating its potential broad-spectrum anticoronaviral activity. Especially, C5N17 racemate (EC50 = 1.5 μM, EC50 = 0.96 μM) displayed significant antiviral activity against nirmatrelvir-resistant SARS-CoV-2 strains (T21I/E166V and L50F/E166V), surpassing the potency of nirmatrelvir (EC50 > 100 μM). Crystallography studies revealed a unique multi-site binding mode. Future efforts will focus on improving metabolic stability and PK profiles.

041. Identification of Small-Molecule Inhibitors of Coronaviruses by Targeting Protein-Protein Interactions in RNA-Dependent RNA Polymerase Complex

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The arsenal of antiviral drugs is currently very limited. It consists of only ~90 molecules primarily targeting the activity of viral enzymes, which leaves other biological features vastly underexplored. This might complicate the rapid and effective answers to emerging viruses. For example, the ongoing COVID-19 pandemic highlighted the critical needs for the proactive identification of antiviral drugs with complementary modes of action and broad-spectrum activity. Here, to develop pan-coronavirus inhibitors that potentially reduce the frequency of adaptive evolution compared to enzymatic drugs, we have targeted a conserved protein-protein interaction (PPI) within the core SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) complex. We have first demonstrated that the Nsp12-Nsp8 interface is a better target than Nsp12-Nsp7 for direct PPI inhibition. Using advanced interactomic tools in human cells, we then subjected the Nsp12-Nsp8 target to ~18,000 candidate inhibitors. To select for interaction-specific compounds, we performed a parallel counter-screen using an irrelevant PPI. This led us to identify

three specific Nsp12-Nsp8 inhibitors with minimal cytotoxicity and potent antiviral activity These new molecules significantly decreased viral replication when combined to Remdesivir, which highlights the potential for combinatorial therapies. Importantly, they are very effective against different SARS-CoV-2 variants, as well as other coronaviruses. Overall, our results position the conserved RdRp complex as a central element to identify small molecules with complementary modes of action to existing drugs. They also shade lights on strategies to improve pandemic preparedness.

042. A CRISPR/Cas9 Genetically Engineered Organoid Biobank To Study Coronavirus Host Factors

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Rapid identification of host genes essential for virus replication may expedite the generation of therapeutic interventions. Genetic screens are often performed in transformed cell lines that poorly represent viral target cells in vivo, leading to discoveries that may not be translated to the clinic. Intestinal organoids (IOs) are increasingly used to model human disease and are amenable to genetic engineering. To discern which host factors are reliable anti-coronavirus therapeutic targets, we generated mutant clonal IOs for 19 host genes involved in coronavirus biology, focusing on host proteases involved in mediating coronavirus entry. In IOs, entry of the early 614G SARS-CoV-2 strain does not require the endosomal Cathepsin B/L proteases, but specifically depends on the cell surface protease TMPRSS2. Other host factors were not essential. Based on cell line data it is now widely accepted that Omicron variants use TMPRSS2 less efficiently and instead use cathepsins, but these findings have yet to be verified in more relevant cell models. Although we could confirm efficient cathepsin-mediated entry for Omicron in cell lines, CRISPR-edited IOs showed that entry of Omicron variants still relied on TMPRSS2, and not cathepsin B/L. SARS-CoV and MERS-CoV similarly depended on TMPRSS2. We verified our data using inhibitor experiments in airway organoids. These findings underscore the relevance of combining organoid models with CRISPR/Cas9 in virus research, identify TMPRSS2 as an attractive pan-coronavirus therapeutic target, and demonstrate that an IO knockout biobank is a valuable tool for the discovery and validation of host-directed antiviral strategies.

043. Identification of Novel Host Proteins that are Associated with Macrophage Control of Influenza A Virus Replication

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Airway macrophages (MΦ) are an important component of the innate host-defence against influenza A virus (IAV). In contrast to airway epithelial cells, MΦ limit seasonal IAV infection through "abortive replication" such that infectious virus particles are not released from infected cells. Our ongoing research program aims to investigate the consequences of abortive IAV replication in MΦ in the context of contributing to effective innate host immunity. However, some highly pathogenic avian influenza virus (HPAI) strains replicate productively in MΦ, which has been mapped to the viral hemagglutinin (HA) protein. We used reverse genetics (RG) H5N1 viruses containing 6 genes from seasonal IAV plus HA/NA genes from HPAI H5N1 (but lacking the multibasic cleavage site (MBCS)) to identify strains that productively or abortively replicate in MΦ. We leveraged this key observation in an innovative proteomics approach to identify MΦ proteins that differentially bound to HA proteins associated with productive or abortive IAV replication. We expressed V5-tagged HA proteins associated with productive and abortive

replication in MΦ and co-precipitated each HA and associated MΦ proteins. Mass spectrometry and proteomics analysis identified (i) proteins that bound both HA and (ii) proteins bound only HA associated with productive replication. We use in vitro protein overexpression and knockdown approaches to validate the ability of novel host proteins to modulate the replication of influenza A virus.

044. A Mouse Model of Human Parainfluenza Virus Type 3 Infection to Study Prophylactic and Therapeutic Modalities

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Human parainfluenza virus type 3 (HPIV3) causes upper and lower respiratory tract infections in particular in young children, the elderly and immunocompromised individuals. There are no vaccines or antiviral drugs available. In an effort to establish a robust small animal infection model, we explored HIPV3 replication in various mouse strains [i.e. Balb/C, SCID, C57bl/6, Ifnar-/-, and AG129 (Interferon α/β/γ R-/-)]. AG129 mice supported viral replication most efficiently. Intranasal inoculation resulted in replication in both the upper and lower airways with peak viral loads on day 1, 2 and 3 post infection. Using RNAscope, infection of the epithelium was demonstrated, in particular of the club and ciliated cells. From day 5 p.i. onwards, infection resulted in marked lung pathology characterized by peri-vascular and peri-bronchial inflammation, bronchopneumonia and hyperplasia of pneumocytes. GS-441524, the parent nucleoside of remdesivir, was used to validate the model for antiviral studies. Oral treatment with GS-441524 (50mg/kg) reduced infectious virus titers in lungs to undetectable levels and resulted in histologically normal lungs. Intranasal treatment reduced viral loads in both lungs and nasal mucosa. Even a single oral prophylactic dose of GS-441524 completely prevented HPIV3 infection. Interestingly, infected mice did not transmit the virus to uninfected sentinels housed in the same cage. In conclusion, we established, to the best of our knowledge, the first robust mouse HIPV3 infection model and validated the model for antiviral studies. This model is also well suited to assess the efficacy of neutralizing antibodies and of vaccine candidates.

045. Engineering protease-resistant peptides to inhibit human parainfluenza viral respiratory infection

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The lower respiratory tract infections affecting children worldwide are in large part caused by the parainfluenza viruses (HPIVs), particularly HPIV3, along with human metapneumovirus and respiratory syncytial virus, enveloped negative-strand RNA viruses. There are no vaccines for these important human pathogens except those recently released for respiratory syncytial virus, and existing treatments have limited or no efficacy. Infection by HPIV is initiated by viral glycoprotein-mediated fusion between viral and host cell membranes. A viral fusion protein (F), once activated in proximity to a target cell, undergoes a series of conformational changes that first extend the trimer subunits to allow insertion of the hydrophobic domains into the target cell membrane, and then refold the trimer into a stable postfusion state, driving the merger of the viral and host cell membranes. Lipopeptides derived from the C-terminal heptad repeat (HRC) domain of HPIV3 F inhibit infection by interfering with the structural transitions of the trimeric F assembly. Clinical application of this strategy, however, requires improving the in vivo

stability of antiviral peptides. We show that the HRC peptide backbone can be modified via partial replacement of α-amino acid residues with β-amino acid residues to generate α/β-peptides that retain antiviral activity but are poor protease substrates. Relative to a conventional α-lipopeptide, our best α/β-lipopeptide exhibits improved persistence in vivo and improved anti-HPIV3 antiviral activity in animals.

046. Aptamer-based Glycoprotein Broad-spectrum Blocking Strategy Inhibits Respiratory Syncytial Virus Infection

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RSV disease generates an alarming proportion of overall deaths globally, with 1 in 50 deaths among children aged 0-60 months attributable to RSV. Natural infection with RSV does not produce lasting immunity and is prone to repeated infection with the same subtype of RSV. To date, only Bavirin spray, Synagis (pavizumab) and Beyfortus (nirsevimab), approved by the FDA, are available for RSV treatment. There is still a lack of effective and rapid response drugs and treatment strategies to curb RSV infection. Herein, we demonstrated an Aptamer-based Broad-spectrum blocking strategy by engineering aptamers' binding to the region on RSV Glycoprotein, leading to block RSV infection. We identified a group of high affinity (KD=0.016 92 nM) and specific ssDNA aptamers targeting RSV Glycoprotein in salivate via CE-SELEX. The identified aptamers were tightly bound to the Glycoprotein of RSV A long and A2 train to form stable complexes, and were able to detect the added RSV pseudoviral in saliva (detection limit 254 TU/ml) with good linearity (R2=0.998). Such aptamer-based therapeutics exhibited potent antiviral activity against both the authentic RSV A long strain and A2 train with EC50 values at nanomolar. The affinity evaluation also evidenced that these aptamers exhibit strong affinities for Glycoprotein of pan-RSV (long, A2 and 9320 train). In conclusion, we have identified six aptamers with a high broad-spectrum anti-RSV activity, which could potentially serve as an effective strategy for preventing infections by RSV and addressing the ongoing global health threat.

047. Inhibition of Rhinovirus Infection in Differentiated Primary Human Bronchial Epithelial Cells by Nanoparticle-Encapsulated Small Interfering RNA

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Introduction: Rhinovirus (RV) infections precipitate exacerbations of chronic airway diseases, bronchiolitis, & pneumonia. Despite this, there are no approved RV antivirals. Therapeutic development has been hindered by the high number of genetically distinct RV subtypes (>174). RNA interference (RNAi) is a biological process in which small non-coding RNAs (e.g. siRNA) guide sequence-specific gene silencing. RNAi could be used to directly target the highly conserved 5′ untranslated region (UTR) of the RV genome. Lipid nanoparticle (LNP) encapsulation of siRNAs can bypass in vivo nucleic acid degradation, enhancing siRNA delivery & stability. We hypothesised that LNP-siRNAs targeted towards the RV 5′ UTR would inhibit viral replication, providing broad spectrum protection against RVs.

Methods/Results: Sequence alignment & siRNA design tools were used to select siRNAs targeting conserved regions of the RV genome. siRNAs were encapsulated using the NanoAssemblr platform, producing particles of consistent size (~95nm) with high encapsulation efficiency (>96%). Differentiated primary human bronchial epithelial cells were treated with LNP-siRNAs followed by inoculation with RV. We assessed viral replication by qPCR (RV RNA) & infectious virus endpoint dilution assay (TCID50). Type I/III interferon (IFN) expression was assessed by qPCR. LNP-siRNAs significantly (one-way ANOVA, $p>0.05$) reduced RV titre 48-hours post-inoculation. Type I/III IFN mRNA levels were not affected by treatment.

Conclusion: We show that LNP-siRNAs targeted towards the RV 5′ UTR significantly reduces the viral titre of RV-A1 in vitro. We next aim to assess the RV antiviral capacity of LNP-siRNAs in vivo.

048. Development of Small Molecule Entry Inhibitors as Novel Therapeutics against Influenza Viruses

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Seasonal or pandemic flu caused by influenza A viruses (IAV) is a major public health concern due to the high morbidity and significant mortality, especially in high-risk population groups. Although there are several classes of drugs targeting different viral proteins, emergence of drug resistance strains calls for a continuous search for new drug candidates that can be used alone or in combinations. We have been developing small molecule inhibitors targeting IAV hemagglutin (HA) proteins and studying mechanism of action (MOA) of these inhibitors. Since viral HA proteins are classified into two disitinctive groups (group 1 and group 2), and small molecule inhibitors generally block either group 1 or group 2, but not both, we are developing two classes of inhibitors specifically tageting either group 1 or group 2 HA proteins. Toward this goal, via iterative medicinal chemistry and evaluation, we created different series of highly potent anti-IAV agents with excellent in vitro ADMET and druglike properties. Furthermore, our most advanced and effacious in vivo lead compound possesses excellent oral bioavailability and works synergistically with the clinical drugs targeting other viral proteins. These new inhibitors are promising drug candidates against IAV infections. Through an integrated structural biology, biophysical, and molecular biology approach, we showed how these inhibitors interact with HA proteins, and these studies provide new insights on rational drug design and lead optimization.

050. The B Cell Repertoire in Multiple Sclerosis Reveals Molecular Mimicry between EBV EBNA1 and GlialCAM

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Infection with Epstein-Barr virus (EBV) is highly associated with multiple sclerosis (MS). Molecular mimicry of EBV antigens and antigens of the central nervous system (CNS) has been hypothesized to be a driver of neuroinflammation, but mechanistic evidence is scarce. To identify anti-viral antibodies in MS, we single-cell sequenced the antibody repertoire of B cells in the cerebro-spinal fluid (CSF) and blood of MS patients and identified clonal expansions of activated B cells that carry the hallmarks antigen-specific activation. A selection of these antibodies were tested against a spectrum of viruses implicated in MS pathogenesis. One antibody was identified that binds the EBV transcription factor EBNA1 at an epitope known to elicit high antibody reactivity in MS patients. We demonstrate that this EBNA1-binding antibody cross-reacts to the glial cellular adhesion molecule GlialCAM. We provide structural evidence for the evolution of GlialCAM-reactivity from an unmutated B cell binding only EBNA1. Cross-reactivity between the two antigens is facilitated by a post-translational modification of GlialCAM. EBNA1-GlialCAM cross-reactive antibodies are more prevalent in MS patients than in healthy controls. EBNA1 peptide immunization aggravates the mouse model of MS. Together, our results suggest that EBNA1-reactive antibodies can cross-react with the CNS-specific membrane protein GlialCAM and induce neuroinflammation, thereby exacerbating MS. Our results provide a long sought mechanistic link for the association between MS and EBV.

051. From Target to Treatment

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Notwithstanding the increased use of antivirals with the advent of SARS-coV-2, there remain few effective drugs available for treatment of DNA viruses and even fewer for RNA viruses. In my talk I will review work in my group looking at how compassionate-use experimental medicine studies in children with life-threatening RNA viral infections has provided insights into how we might optimise putative antiviral drugs to achieve improved outcomes. The in vivo studies are underpinned by a programme to develop biomarkers of antiviral drug efficacy, to better understand the impact of antiviral drugs in the absence of complete viral clearance and to explore the use of combination therapies. In the final part of my talk, I touch briefly on a new programme of work starting in my lab to better understand how epithelial-tropic viruses including members of the herpesvirus, papillomavirus and pox virus families appear to make use of the same specialised keratinocyte signalling pathways for replication. The results provide opportunities for exploring the possibility of developing new, potentially broad spectrum antiviral drugs.

052. Medicinal Chemistry Optimization and Therapeutic Efficacy of 2-Pyrrolidinoquinazolinones in Lethal Murine Models of Venezuelan and Eastern Equine Encephalitis Viruses

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Prevention and treatment of severe neurological disease caused by the mosquito-borne alphaviruses, Venezuelan (VEEV) and eastern equine encephalitis viruses (EEEV), is challenging due to the lack of effective therapeutics. We recently disclosed the discovery of BDGR-49, a 2-pyrrolidinoquinazolinone, that demonstrated complete protection against VEEV infection in lethal mouse models. Further optimization resulted in an analog, BDGR-164, which features replacement of the BDGR-49 nitro-aryl toxophore, improvements in solubility, microsomal stability, and the maximum tolerated dose, while also exhibiting suitable bioavailability and brain exposure. Using a lethal, subcutaneous VEEV TrD infection mouse model, complete prophylactic (treatment -2h prior to viral challenge) and therapeutic (treatment +1, +2, +4 days post infection, dpi) protection was observed in mice given 24 mg/kg of BDGR-164 twice daily for 8 days, starting on the day of treatment. Clinical signs were similar among VEEV-infected controls and the +4 day treatment group, but treated mice continued to improve through 21 dpi. Brain viral titers at 4 dpi were reduced to non-detectable levels in 75% (+1, +2 day cohorts) or 100% (-2h, +3, +4 day cohorts). In a lethal, subcutaneous EEEV FL93-939 infection mouse model, > 90% survival was observed for infected mice given 24 mg/kg of BDGR-164 twice daily for 8 days with treatment starting as late as +3 dpi. BDGR-164 treatment alone did not induce a Type I interferon response. The structural evolution of the scaffold and associated data will be discussed, highlighting a milestone achievement in the development of an effective VEEV and EEEV inhibitor.

053. Design, Synthesis, and Lead Optimisation of Piperazinyl-Pyrimidine Analogues as Potent Small Molecules Inhibitors of Chikungunya Virus

Now virtual poster

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The worldwide re-emerge of the Chikungunya virus (CHIKV), its high morbidity, and the lack of an available vaccine or antiviral treatment make the development of a potent CHIKV inhibitor highly desirable [1]. Therefore, an extensive high-throughput screening (HTS) was conducted, which resulted in the discovery of a promising novel hit compound 1a (Figure 1) [2]. Comprehensive lead generation and optimisation were performed, focusing on optimising their antiviral activity and crucial ADMET properties. Therefore, 137 analogues were designed, synthesised, and tested for their antiviral activity and physiochemical characteristics. Two synthesis routes were established, enabling the synthesis of the desired piperazinyl-pyrimidine analogues in high yield and short synthesis time. Through two extensive structure-activity relationship studies (SAR), key chemical features for potent anti-CHIKV inhibition were revealed and analysed. Further, the analogues were screened for their aqueous solubility, lipophilicity, possible hERG channel interactions, cytotoxic characteristics in three cell lines (CaCo-2, HEK-293, and A459), and their viral profile. Moreover, a cross-resistance study confirmed the viral capping machinery (nsP1) as the viral target of the CHVB series. In addition, a structure-metabolism relationship study (SMR) was performed with the most promising 55 analogues by assessing their metabolic stability in human liver microsomes (HLMs). The compounds showed an excellent safety profile, favourable physicochemical characteristics, and the required metabolic stability. This research study identified 31b and 34 as potent, safe and stable CHIKV inhibitors (Figure 1) [3].

054. Suicidal capsid protease from O'nyong'nyong virus: unveiling the inhibitory potential of indole derivatives

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Climate change is expanding the geographical reach of neglected tropical diseases, including those caused by re-emerging arthritogenic alphaviruses such as O'nyong'nyong virus (ONNV). Studies on alphaviruses showed the importance of capsid protease (CP) in the virus replication cycle making it a promising drug target. Interestingly, as CP performs the first cleavage event, its C-terminal tryptophane remains buried in the active site pocket, blocking the access to other substrates and inactivating the protease. The goal of the project is to develop effective CP inhibitors capable of blocking its auto-proteolytic activity.
So far, we optimized expression and purification of the autoinhibited ONNV CP and its active variants deprived of C-terminal tryptophane (thereby preventing suicidal cleavage) and assessed their enzymatic activity. Furthermore, we solved a high-resolution structure of ONNV CP in its active form, presumably encountered during the polyprotein synthesis, which was used for the design of indole derivatives as potential CP inhibitors. A hit indole compound, obtained by random screening, displayed micromolar activity on ONNV CP in enzymatic activity assays and exhibited a significantly higher binding affinity than indole itself. It will serve as starting point for lead optimization. To test the inhibitory potential of lead compounds, we developed a model, which is human fibroblast-like synoviocytes infected with ONNV.

Our results set up a platform for designing indole derivatives capable of hindering ONNV replication by targeting ONNV CP and should pave the road for the development of a broad-spectrum antiviral strategies against arthritogenic alphaviruses.

055. Treatment with 6MMPr potentiates the activity of favipiravir in a hamster model of yellow fever

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Yellow fever virus (YFV) continues to cause significant morbidity and mortality in areas of endemicity despite the availability of an effective vaccine. There is a need for an antiviral that is effective against YFV. Favipiravir is active against a wide range of viruses, including YFV in cell culture and in animal models. It was approved for clinical use in Japan but has shown toxicity in clinical trials and has not been approved for use in the United States. The compound 6-methylmercaptopurine riboside (6MMPr) has been shown to have synergistic activity with favipiravir in cell culture by increasing the amounts of ribosylated favipiravir triphosphate, thus reducing the effective dose of favipiravir. A suboptimal dose of favipiravir in combination with 6MMPr was effective in a hamster model of yellow fever (YF) when administered just prior to virus challenge. Significant improvement in disease parameters, as compared with placebo treatment, was observed with favipiravir and 6MMPr combination treatment, while monotherapy with either favipiravir or 6MMPr was not significantly different from placebo. This included an increase in survival and reduced viral titer in animals treated with combination therapy. We confirmed the activity of the combination when administered at the time of virus challenge and demonstrated activity when combination therapy was administered beginning 2 days after virus challenge. Combination treatment with 6MMPr could lower the dose of favipiravir needed to treat acute viral disease and could potentially reduce the toxic effects in vivo. [This work was supported by HHSN272201700041I/Task A51 from the NIAID, NIH]

056. Cellular and Molecular Mechanisms of Arboviral Immunity

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Chikungunya is a neglected tropical disease caused by infection with the chikungunya virus (CHIKV), an alphavirus transmitted by mosquitoes. This disease is typically self-limiting and characterized by common symptoms such as myalgia, fever and rash. However, it is capable of causing devastating long-term effects in rare cases, particularly in new-borns and the immunocompromised. It remains important to identify the specific immune cell populations involved in CHIKV infection, clarifying the mechanisms of host-virus interaction and opening new avenues for immunological therapeutics against chikungunya. I will be discussing various approaches that have been used to decipher insights into the mechanisms of CHIKV immunopathogenesis that have opened new avenues for the development of anti-CHIKV immunotherapeutics targeting the pro-inflammatory macrophages.

057. Development and mechanism of novel Diphyllin derivatives against Ebola virus infection

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Filoviruses are a family single-stranded, non-segmented, negative sense RNA viruses that are often associated with sever hemorrhagic disease. Ebola virus (EBOV), a filovirus associated with high mortality, continues to cause outbreaks in Africa. These devastating endemics emphasize the need for potential treatments for this priority pathogen and a greater understanding of its interaction with the host cell environment. Currently, no small molecule inhibitors have been approved for treatment of filovirus disease. Our group has previously shown that diphyllin, a natural-product small molecule, inhibits Ebola infection at a cell entry step with an EC50 of approximately 1 μM. We have since identified new diphyllin derivatives with low nanomolar potency against EBOV infection with improved, lower cytotoxicity in both stable cell lines and primary human macrophages, a major target cell type for EBOV infection. To gain a better understanding of target specificity and mechanism, we have generated cells lacking expression of isoforms of ATP6V0A, a transmembrane component of the V-ATPase multi-subunit enzyme which was previously reported be a target of diphyllin and is necessary for acidification of endosomes, as well as other potential targets. We find that isoform ATP6V0A2 is a key contributor for diphyllin's activity against EBOV. Furthermore, we show that these targets have varying susceptibility toward derivatives, suggesting the possibly of tuning diphyllin derivatives to specific targets. We demonstrate that our novel derivatives of diphyllin may be promising therapeutics for Ebola virus disease, potentially addressing the gap in treatment options for this severe disease.

058. Identification and evaluation of novel Lassa virus entry inhibitors using computational counter screening and chemical informatics

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Lassa virus (LASV) is a hemorrhagic fever arenavirus of significant public health concern, infecting up to 300,000 people per year. LASV enters host cells by binding of the virus glycoprotein (GP) to a-dystroglycan at the cell surface followed by a receptor switch to LAMP1 within the endosome. Despite numerous efforts to identify therapeutics that inhibit cell entry, a clinically approved small molecule LASV inhibitor has not been reported. To address this gap, we developed a computational strategy to identify specific inhibitors of GP-mediated virus entry based on a previous screen of over 350,000 small molecules performed by our group, comprising the NIH MLPCN library, with data deposited into PubChem. We developed datamining techniques to select for small molecules based on potency and to remove promiscuous compounds with widespread activity in other assays in the database, allowing prioritization of hits by potency as well as mechanism specificity. Cheminformatics was then used to select compounds with diverse chemical scaffolds not previously reported. Each were evaluated for inhibition of pseudotyped viruses bearing LASV, Junín and vesicular stomatitis virus GPs and then wild type LASV at BSL4. This identified candidates displaying high selectivity and sub-micromolar potency for LASV. Preliminary mechanistic evaluation suggests disruption of GP attachment to a-dystroglycan and prevention of glycosylation of host receptors critical for binding. Overall, our computational approach accelerates identification and evaluation of potent antivirals by prioritizing candidates with minimal off-target effects, expediting their progression toward therapeutic development.

059. Identification of a Macrocyclic Compound Targeting the Lassa Virus Polymerase

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Lassa virus (LASV) is endemic to West Africa, where seven genetically diverse clades have been identified. There are no approved vaccines or therapeutics to prevent or treat LASV infections; to identify compounds with anti-LASV activity, we conducted a cell-based compound screening campaign at Biosafety Level-4. Almost 60,000 compounds, including nearly

10,000 macrocyclic compounds, were tested for their ability to inhibit the growth of an infectious, recombinant reporter LASV based on a clade IV sequence. Hits from this screen included a family of structurally related macrocycles. The most potent of these, Mac128, had a sub-micromolar EC50 against the reporter virus, also inhibited the wild type clade IV virus and reduced viral titers by more than four orders of magnitude. Time-of-addition studies, as well as assays recapitulating individual steps in the viral life cycle, suggested that Mac128 acted at the level of virus replication; LASV glycoprotein-dependent entry and Z-protein budding were unaffected, but replication of the LASV minigenome was blocked. We found that Mac128 was less effective against LASV from clades other than IV and was ineffective against a clade II virus. In the minigenome assay system, switching of clade IV and clade II support plasmids was consistent with Mac128 acting primarily at the level of the polymerase. Mac128 is a tool compound for the study of LASV replication and a novel starting point for an optimization campaign with the aim of obtaining a candidate LASV therapeutic.

060. Tribbles Pseudokinase 3 Promotes Enterovirus A71 Infection via Dual Mechanisms

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Enterovirus A71 (EV-A71) is the main pathogen causing hand, foot and mouth disease (HFMD) in children and occasionally associated with neurological diseases such as aseptic meningitis, brainstem encephalitis (BE) and acute flaccid paralysis. We report here that cellular pseudokinase tribbles 3 (TRIB3) facilitates the infection of EV-A71 via dual mechanisms. In one hand, TRIB3 maintains the metabolic stability of scavenger receptor class B member 2 (SCARB2), the bona fide receptor of EV-A71, to enhance the infectious entry and spreading of the virus. On the other hand, TRIB3 facilitates the replication of EV-A71 RNA in a SCARB2-independent manner. The critical role of TRIB3 in EV-A71 infection and pathogenesis was further demonstrated in vivo in mice. In comparison to wild-type C57BL/6 mice, EV-A71 infection in TRIB3 knockdown mice (Trib3+/-) resulted in significantly lower viral loads in muscular tissues and reduced lethality and severity of clinical scores and tissue pathology. In addition, TRIB3 also promoted the replication of coxsackievirus B3 (CVB3) and coxsackievirus A16 (CVA16) in vitro. In conclusion, our results suggest that TRIB3 is one of key host cellular proteins required for the infection and pathogenesis of EV-A71 and some other human enteroviruses and may thus be a potential therapeutic target for combating the infection of those viruses.

061. Generation and Optimization of Bangladesh and Malaysian Recombinant Reporter Nipah Viruses for Antiviral Screening in vitro and Disease Modeling in vivo

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Nipah virus (NiV) causes near-annual outbreaks of fatal encephalitis and respiratory disease in South Asia with a high mortality rate (~ 70%). Since there are no approved therapeutics for NiV disease in humans, the WHO has designated NiV and henipaviral diseases a priority pathogen for research and development. We generated a new recombinant green fluorescent reporter NiV of the circulating Bangladesh genotype (rNiV-B-ZsG), and optimized it alongside our previously generated Malaysian reporter counterpart (rNiV-M-ZsG) for antiviral screening in primary-like human respiratory cell types. Through validating our platform for rNiV-B-ZsG against a synthetic compound library directed against viral RNA-dependent RNA polymerases, we identified and confirmed the sub micromolar activity (EC50 ~ 0.02, selective index > 600) of a hit compound (OTA-4) against wild-type, green fluorescent reporter, as well as a newly constructed, bioluminescent red fluorescent double reporter (rNiV-B-BREP) NiV. Through conducting time of addition, NiV minigenome, and NiV glycoprotein-mediated cell-cell fusion assays we determined that OTA-4 is likely a non-nucleoside replication inhibitor of NiV. We furthermore demonstrated that the reporter rNiV-B-ZsG and rNiV-B-BREP viruses showed comparable pathogenicity to wild-type NiV-B in the Syrian golden hamster model of disease, supporting additional use of these as tools for both pathogenesis and advanced pre-clinical studies in vivo. In sum, we have established robust reporter virus systems for Nipah virus to support high throughput antiviral screening, and as critical tools to further understand mechanisms of disease for targeted therapeutics.

062. Intranasal route to immunity: single dose mucosal delivery of viral replicon particle vaccine protects uniformly against lethal Nipah virus challenge in African green monkeys

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Nipah virus (NiV) is a highly lethal zoonotic virus causing near annual high-mortality outbreaks in areas of Southeast Asia, India, and Bangladesh. We previously developed a novel NiV viral replicon particle (VRP) vaccine based on a recombinant NiV lacking the fusion (F) protein gene (termed NiVΔF). This design restricts NiVΔF to transcriptional and translational processes in the initial host cell entered only, without further dissemination. Importantly, given that NiV is classified as a BSL-4 pathogen, extensive evaluations of NiVΔF in four highly sensitive animal models have revealed no evidence of disease or pathology, confirming its safety profile. Moreover, previous data have shown 100% protection in rodent models of disease. Building on these data, we evaluated intranasal and intramuscular administration in an African green monkey model of lethal disease. Animals were followed closely to evaluate safety post vaccination; again, all clinical parameters remained within normal limits, with no changes noted even on advanced imaging (CT, MRI). In the challenge model, single-dose delivery by either route conferred 100% protection one month after vaccination. Given our previous data indicating that protection can be achieved in the absence of neutralizing antibodies, we performed detailed immunological analyses of humoral and cell-mediated responses in these animals to investigate non-neutralizing mechanisms of protection. Here, we present these data on safety, efficacy, and immunological correlates of protection, advancing knowledge of NiV infection and supporting continued pre-clinical evaluation of the NiV VRP vaccine candidate.

063. Evaluation of Small Molecules as Promising Broad-Spectrum Anti-Filoviral Agents

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Ebola and Marburg filoviruses (EBOV and MARV, respectively) are two of the most pathogenic RNA viruses. EBOV and MARV infection results in viral hemorrhagic fever (VHF), a syndrome that in severe cases leads to multiorgan failure and internal bleeding. In addition to being highly contagious, these filoviruses cause high mortality rates (23-90%). Currently, two vaccines are FDA-approved against EBOV disease, however, they only display efficacy against EBOV Zaire, while recent EBOV outbreaks have been caused by EBOV Sudan, emphasizing the need for broad-spectrum vaccines and antivirals. We conducted a high throughput screen for ebolavirus entry inhibitors and identified a hit designated as CBS1118, with EC50 < 10 μM against both EBOV and MARV. Structure-activity relationship studies on CBS1118 led to the development of compounds with improved selectivity and potency against pseudotyped EBOV and MARV, further confirmed in an inhibitory assay against infectious viruses. Our lead compound, MWAC-0001911, shows nanomolar potency against pseudotyped MARV and multiple EBOV strains, including Sudan, with a high specificity over unrelated pseudotyped viruses, including SARS-CoV-2, used in the counter-screen. Biophysical and structural studies indicate that MWAC-0001911 binds to a region close to the toremifene-binding site in the EBOV glycoprotein (GP), providing structure-based optimization strategies for our lead inhibitors. Pan-filoviral therapeutics in combination with vaccines may be the key to the treatment and prevention of deadly filovirus outbreaks.

064. Small molecule antiviral candidates for Rift Valley Fever

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Rift Valley fever (RVF) is a mosquito-borne and important zoonotic disease and has potential to cause human outbreak and pandemic with global climate changing and warming. So far, no commercial fully licensed vaccines and effective antivirals are available in the USA, and European and Asian countries. To identify effective antivirals for RVF, we have screened the libraries containing approximately 26,136 chemical compounds that are predicted to cross the blood-brain barrier using a developed high-throughput assay based on RVFV MP12 vaccine strain expressing the Renilla luciferase. We have identified 239 candidates that are effective to inhibit RVFV replication. Thirty-five compounds were selected for further analysis to determine their efficacy inhibition of RVFV replication. Our further analysis reveal that eight compounds show a low cytotoxicity and a low IC50 and are promising by using the plaque assay. Furthermore, two compounds (WMA-RV1 and WMA-RV7) show to inhibit the polymerase activity in contrast to the positive control T705 that has been identified to be an effective inhibitor of viral RNA polymerase. Regarding the underlying mechanisms of these compounds inhibiting the RVFV and their PK and ADME are under investigated.

065. In Silico Tools for Antiviral Research and Future Pandemic Forecasting

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Herein, we provide an overview of the key computational methods and their applications for antiviral research. These include ligand-, structure-, and knowledge-based approaches. We will briefly overview the methodology behind each approach as well as provide examples of their use in (i) antiviral drug discovery and repurposing; (ii) identification of conserved viral proteins as targets of broad-spectrum antivirals; (iii) identification of host targets to prevent viral entry and replication; (iv) development and mining of knowledge sources to develop broad-spectrum antivirals; (v) identification of synergistic combinations of antivirals; (vi) impact of different factors on the dynamics of scientific community's response to several epidemics; and (vii) extensive knowledge mining to forecast future viral disease outbreaks as well as the regions of the world most likely to be impacted by future outbreaks. We will also reflect on the importance of open science and multidisciplinary collaboration for the development of successful in silico models. We posit that truly impactful computational tools must deliver actionable, experimentally testable hypotheses enabling the discovery of novel drugs and drug combinations, and that open science and rapid sharing of research results are critical to accelerate the development of novel, much needed therapeutics.

066. Hepatitis B Virus cccDNA Biosynthesis, Epigenetics, and Antiviral Development

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Chronic hepatitis B virus (HBV) infection remains a significant public health burden worldwide. HBV covalently closed circular DNA (cccDNA) is essential to the virus life cycle by serving as the persistent form of viral genome and transcription template, its complete elimination or inactivation during chronic infection is considered critical to a cure but has not been achieved by current antivirals. cccDNA is formed through a DNA repair process of the viral genomic relaxed circular DNA (rcDNA) by hijacking the host DNA repair machinery, and once formed, cccDNA exists in a stable episomal minichromosome decorated with host histones and nonhistone proteins. Accumulating evidence suggests that epigenetic modifications of cccDNA contribute to viral replication and the outcome of chronic HBV infection. Furthermore, HBV X protein (HBx) is known as a cccDNA transcription activator and essential for maintenance of cccDNA at transcriptionally active epigenetic configuration. In my presentation, together with the recent work from my lab, I aim to delineate recent advancements in HBV cccDNA formation and epigenetics research, elucidating the virus-host interplay between these processes. Furthermore, I will discuss the antiviral strategies and approaches that target cccDNA for eradication and/or inactivation, holding promise for achieving a cure of chronic HBV infection, particularly when integrated into combination therapy regimens.

067. Discovery of a pan-genotype hepatitis E virus replication inhibitor exerting potent in vivo efficacy

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The hepatitis E virus (HEV) constitutes a substantial public health burden with ~20 million human infections annually, including 3.3 million symptomatic cases. HEV causes acute or chronic viral hepatitis in humans. It is fecal-orally transmitted through contaminated drinking water or undercooked meat. The only available vaccine is exclusively available in China and Pakistan,

and is not effective against all HEV genotypes. Treatment options are limited with the off-label use of ribavirin. Appropriate treatment options for HEV-infected pregnant women and immunocompromised patients are currently lacking, underscoring the pressing requirement for safe and efficacious pan-genotype therapies to combat HEV infections.

We report on the identification of JNJ-9117 that exerts nanomolar, pan-genotype antiviral activity against HEV in different cell types, including primary human hepatocytes. JNJ-9117 is a nucleoside analogue and is assumed to target the viral polymerase as its 5′-triphosphate form. JNJ-9117 has a promising pharmacokinetic and safety profile in rats and dogs. In addition, it exhibited strong antiviral activity in the nude rat HEV infection model. Remarkably, starting treatment 5 or 10 days post-infection led to a rapid and significant reduction in viral load within 4 days. Moreover, at the highest dose, suppression of viral RNA levels in feces and liver tissue was effectively maintained until the end of the study at day 17 or 22 after treatment discontinuation. An additive effect was observed in vitro when combining JNJ-9117 with ribavirin. Altogether, these results illustrate the potential of JNJ-9117 as a promising candidate for the treatment of HEV infections.

068. Functional evaluation and mode of action of a novel non-nucleoside drug inhibiting the replication of Hepatitis Delta virus

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Co-infection with HDV (on HBV) significantly increases the risk of progression to cirrhosis and liver cancer. Since the HDV genome does not encode a specific enzyme, it is impossible to develop a conventional direct-acting antiviral, and there is still no satisfactory treatment.

In this context, we have initiated a screening program to isolate small molecules capable of inhibiting the replication and propagation of HDV. These efforts have led to the identification of a new non-nucleoside inhibitor of both HBV and HDV. This molecule, called APY1, belongs to a chemical series based on an alkoxypyrazole central core. The antiviral activity of APY1 has been established in cultures of HepaRG cells and primary human hepatocytes (PHH) co-infected with HBV and HDV. While APY1 reduces all virological parameters of HBV in vitro (secretion of HBsAg/HBeAg, intracellular viral RNA, and virion secretion), this molecule most efficiently inhibits the production of infectious HDV particles with an IC50 in the micromolar range. We have shown that while the secretion of HBsAg is not quantitatively inhibited by APY1, this molecule disrupts the glycosylation pattern and folding of HBsAg so that the antigenic loop is not detected anymore by conformation-specific antibodies. This explains to the loss of infectivity of the produced HDV particles. In order to optimize its properties, more than 350 analogs of APY1 have been synthetized and their evaluation showed a strong correlation between the ability of molecules to alter the folding of HBsAg and the loss of infectivity of the HDV particles.

We are currently further elucidating the MoA of APY1 and optimizing its pharmacologocal properties.

069. Discovery of First-in-Class Hydrophobic Tagging (HyT)-based Degraders of HBV Core Protein

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Interfering with the assembly of hepatitis B virus (HBV) capsid is a promising approach for treating chronic hepatitis B (CHB) [1]. Anti-HBV agents with novel mechanisms of action are urgently required to overcome the challenges of drug resistance, and targeted protein degradation (TPD) strategy constitutes excellent candidate for this purpose. Herein, the first degradation of HBV core protein (HBc) using small-molecule degraders developed by hydrophobic tagging (HyT) technology to effectively combat HBV was reported [2]. The structure-activity relationship (SAR) results revealed that compound S7 with adamantyl group displayed excellent inhibitory activity (EC50 = 0.46 μM), and substantial degradation ability with a time- and dose-dependent manner. Mechanistic studies demonstrated that the Hsp70-mediated, and autophagic lysosome pathway were the potential drivers of S7-induced HBc degradation. Furthermore, molecular dynamics simulation results showed that the adamantyl group extended on the surface of the protein, which may help S7 to interact with specific molecular chaperones to achieve HBc degradation. This proof-of-concept study underscores the potential of HyT-mediated targeted protein degradation in HBc, presenting an innovative and highly promising avenue for the discovery of HBV drugs.

[1] Shao X, Xu S, Wan X, et al. Medicinal chemistry strategies in the discovery and optimization of HBV core protein allosteric modulators (2018-2022 update). Chin Chem Lett. 2023, 34 (11): 108349.
[2] Xie S, Zhu J, Li J, et al. Small-molecule hydrophobic tagging: a promising strategy of druglike technology for targeted protein degradation. J. Med Chem. 2023, 66: 10917-10933.

070. Proof of Concept: Exploring the Therapeutic Potential of G3BP1 Targeted Degradation Against Norovirus Infection

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Human norovirus is a major cause of gastroenteritis worldwide, for which no specific antiviral therapeutics exist to date. Norovirus propagation in the host cell is highly dependant on the expression of G3BP stress granule assembly factor 1 (G3BP1), a multifunctional protein involved in the assembly of RNA-protein condensates under cellular stress conditions. We have previously shown that G3BP1-knockout cells fail to replicate both human and murine norovirus (MNV), therefore, we hypothesize that degradation of G3BP1 could be a promising antiviral strategy. Targeted protein degradation has recently emerged as a novel approach by which proteolysis of specific targets is induced via the proteasome. Targeted protein degradation can be achieved by using Proteolysis-targeting-chimeras (PROTACs), heterobifunctional molecules that recruit specific E3 ligases to a protein of interest. Here, we sought to develop a cellular model in which the antiviral activity of PROTACs targeting G3BP1 (fused to Halotag) can be evaluated. First, we found that MNV replication is restored in G3BP1-knockout cells complemented with Halotag-G3BP1. Furthermore, treatment of these cells with HaloPROTAC-3 led to Halotag-G3BP1 degradation by 48 hours (DC50 463 nM). Currently, we are testing the degradative capacity of a panel of HaloPROTACs with diverse chemical warheads; as well as their ability to reduce MNV replication by different methods. In sum, we have developed a cellular model in which G3BP1 can be efficiently targeted and degraded using HaloPROTAC-3. We are using this model to investigate, as a proof of concept, the therapeutic potential of G3BP1 degradation in the context of norovirus infection.

071. Protease Inhibitor Activity Varies Between Genogroup I and Genogroup II Noroviruses

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Human norovirus (HuNV) protease (Pro) is critical for processing the viral polyprotein and an attractive target for antiviral drug development. Broad spectrum antivirals that target multiple viral strains with similar efficacy within the diverse norovirus genus are required for treatment of infections. Current protease drug development and structural characterisation has focussed on genogroup (GI) viruses, but genogroup II (GII) viruses represent a significant proportion of the norovirus gastroenteritis cases worldwide. We have tested a series of known peptidomimetic protease inhibitors with a variety of chemical moieties against both GI and GII Pro. The results show that antivirals targeting GI Pro are also capable of inhibiting GII Pro, although in all instances they were less potent against GII Pro. Of the inhibitors tested, NV-004 had an IC50 of 0.2 μM and 0.4 μM with GI and GII Pro respectively. To understand the structural basis for inhibition, the crystal structure of GII Pro at 2.79 Å and at 1.83 Å with NV-004 bound in the active site was determined. Pro was observed in both open and closed conformations based on the orientation of the Arg 112 side chain, potentially hindering access of inhibitors to the active site in GII. The structure of GII Pro with NV-004 reveals changes in the active site cleft and substrate binding pockets. This is the first inhibitor bound GII Pro structure and will aid in structure-guided antiviral development that targets both GI and GII Pro.

072. Towards an HIV Cure: Novel Approaches to Reduce and Control the Reservoir

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Despite the great success of antiviral therapy (ART), treatment is life long. The main reason ART is unable to cure HIV infection is the persistence of long lived proliferating latently infected cells, that are primed to survive and escape immune-mediated clearance.

New strategies are therefore needed to reduce the pool of latently infected cells. One approach is to use latency reversing agents that can enhance detection of a latently infected cell by increasing expression of viral proteins. We have recently developed two novel latency reversing agents that are highly potent and HIV specific. These include the delivery of the potent viral transactivating protein Tat and CRISPR activation with HIV-specific guide RNAs. We have successfully delivered both HIV-specific latency reversing agents using mRNA packaged in a novel T-cell tropic lipid nanoparticle that can potently transfect resting T cells. Another approach is to enhance death of latently infected cells, given that these cells overexpress survival proteins, including BCL2. We have recently demonstrated that latently infected cells can be depleted ex vivo and in an animal model with the anti-BCL2 drug venetoclax

Finally, given that it will be impossible to deplete every infected cell, strategies are needed to enhance immune control of any residual replication competent virus. This approach has been successful in animal models with some monkeys truly cured or in sustained remission off ART. We and others have recently completed clinical trials of broadly neutralizing antibodies and anti-PD1 and shown that a subset of participants can indeed control virus replication off ART for a limited period of time.

073. Antivirals Targeting the Conserved HIV-1 TIM-TAM Riboswitch Specifically Reactivate HIV-1 from Latency through Modulating Viral RNA-biology

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Viral RNAs fold into "breathing" RNA structures that impact on viral replication and host defences. We pursued HIV latency reversing agents (LRAs) that target a highly conserved RNA structure overlapping the HIV Tat coding sequence that we called the Tat IRES modulator of tat mRNA (TIM-TAM). TIM-TAM functions as a riboswitch regulating mRNA splicing in the nucleus and translation of Tat mRNA in the cytoplasm, impacting HIV-1 protein output, viral pathogenesis and infectivity, and the switch between productive and latent infection states through modulating production of viral RNA and protein. TIM-TAM riboswitch reporter cells, FlipInFM, were used to screen 114,000 compounds for seven LRA leads. Five leads were BRD4 inhibitors inferior to JQ1, so we pursued the novel amidothiazole lead that specifically reactivated HIV in primary patient cells. Progressive rounds of med-chem increased the potency from EC50=23.6uM to EC50=30.9nM in FlipInFM. The amidothiazoles synergise with JQ1 (+) across three models of latency: FlipInFM dual reporter (Bliss Independence (BI)=0.132), J.Lat T-cell model (BI=0.155) and primary quiescent T-cells (BI=0.196). The HIV TIM-TAM mRNA contains a DRACH motif (AAACU) for m6A modification overlapping a HMGB3 binding site. TIM-TAM RNA lacking m6A activates an IRES-mediated translation from any RNA containing a Tat-coding exon of Tat trans-activator. Inhibitors of the METTL-3 writer of the m6A mark also activate latent HIV. The SAR matches the increases in thermodynamic docking reactivities into the interior of the TIM-TAM helix adjacent to the DRACH site, indicating activity through RNA interactions may disrupt m6A RNA modifications.

074. Mechanisms of HIV-1 Hypersensitivity to Islatravir (4'-ethynyl-2-fluoro-2'-deoxyadeonsine (EFdA))

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Combination antiretroviral therapy (cART) is highly effective at suppressing human immunodeficiency virus type 1 (HIV-1), allowing patients to have an increased quality and length of life. Although cART is highly successful at inhibiting HIV replication, drug-resistant mutations can arise and pose a major threat to the efficacy of these treatments. Therefore, there remains a need to develop novel antivirals, with exceptional interest in long-acting treatments. Islatravir (ISL), is a highly potent antiviral drug candidate currently in phase III clinical trials that has the potential to be a long-acting HIV-1 reverse transcriptase (RT)-targeting inhibitor. ISL inhibits HIV-1 by blocking the translocation of RT; thus, ISL is termed a nucleoside RT translocation inhibitor (NRTTI). Interestingly, the RTF227C resistance mutation, that frequently emerges during therapy with several non-nucleoside RT inhibitors (NNRTIs), was found to make HIV-1 more susceptible to ISL. Why a mutation that is located almost 12 Å from where ISL binds would cause hypersensitivity is not well understood. Here, we determine the biochemical and structural mechanism of RTF227C hypersensitivity to ISL utilizing X-ray crystallography and various biochemical assays. Due to the observed complementary resistance and hypersensitivity profile of certain NNRTIs with ISL, pairing these antivirals may be an optimal combination therapy. Therefore, we also analyzed synergy pairings of ISL with several NNRTIs. Understanding the mechanism of this RTF227C hypersensitivity to ISL is essential in developing optimized combination therapies and for leveraging hypersensitivity mutations to combat drug resistance.

075. HSV-1 Latency is Established in Human Neurons in which Viral Genes are Expressed and Viral DNA is Replicated during the Acute Infection

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Herpes Simplex Virus 1 (HSV-1) infects about 70% of the world population, causing some severe presentations. Pathogenesis relies on lifelong latent infections, in which viral proteins are not expressed and genomes are not replicated, and periodic reactivations. Latency occurs in neurons and is thus studied in post-mortem human samples, animal models, or in vitro infections using viral mutants, isolated neurons, or antivirals. It is generally assumed that latency is established with no prior protein expression or DNA replication and that HSV-1 gene expression and DNA replication is as cytopathic to neurons as to all other cells. However, existing models are not amenable to test this hypothesis. Using neurons differentiated from inducible human progenitor cells, we have developed a model in which wild-type HSV-1 establishes latency spontaneously after replicating in, and spreading between, neurons for 15-20 days; latent genomes are reactivated by stress after 40 days. Using this model, we evaluated the fate of the neurons that support viral gene expression and genome replication. Replicating viral DNA was labelled and analyzed by click chemistry and confocal microscopy. Viral DNA replication was abundant on day 4, subsided by day 10, and was undetected by day 20; afterward, genomes were maintained in the absence of infectious virus. Many neurons in which viral genomes had replicated on days 4-10 were still viable and harbored viral genomes on day 40. The replicated genomes became compacted during latency and some decompacted during reactivation. In summary, a subset of neurons survive HSV-1 genome replication before the viral genomes are silenced and latency is established.

076. Evaluation and Pharmacokinetics of the POM-L-BHDU-MP Prodrug Against Varicella Zoster Virus and Herpes Simplex Virus 1 in vivo

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The bromovinyl-L-dioxolane uridine nucleoside analog, L-BHDU, is highly active against varicella zoster virus (VZV) and herpes simplex virus 1 (HSV1) in cultured cells. It is moderately active against HSV2 (EC50 7 μM). We synthesized the prodrug POM-L-BHDU-MP with a bis(pivaloyloxymethyl) group to improve its pharmacologic properties while retaining antiviral activity (VZV EC50 0.04 μM; HSV1 EC50 0.03 μM; CC50 >100 μM). Here, we evaluated POM-L-BHDU-MP against VZV and HSV1 in skin organ culture and mice, and we studied the pharmacokinetics and distribution. POM-L-BHDU-MP in cocoa butter (0.2% top) prevented VZV or HSV1 spread and was nontoxic to human skin explants. In the NuSkin mouse model, POM-L-BHDU-MP reduced VZV spread via subcutaneous and oral routes (45, 22.4, 11.3 mg/kg) and was well tolerated. In the BALB/c mouse cutaneous flank model, POM-L-BHDU-MP (22.4 mg/kg po) reduced HSV1-induced weight loss, and more studies are underway. Mice were given POM-L-BHDU-MP orally or intravenously and their plasma and organs were analyzed by LC-MS/MS. POM-L-BHDU-MP was rapidly converted to L-BHDU, and the oral bioavailability was high. L-BHDU (22.5 mg/kg po) in plasma reached Cmax of 10 ± 2.5 μg/mL with Tmax of 0.85 h; the half-life was 5-6 h. L-BHDU was distributed in mouse organs, including in the brain and cerebrospinal fluid. The phosphorylated metabolites of L-BHDU were traces in uninfected mice, whereas the di- and triphosphate forms of L-BHDU were 30-50 μM in VZV-infected human skin xenografts, which exceeds the EC90. Overall, POM-L-BHDU-MP is a potent prodrug of L-BHDU, which is a promising nucleotide analog for treating VZV and HSV1 infections.

080. Design and Synthesis of Clickable Photoaffinity Probes for Binding Site Identification on Yellow Fever Virus NS4B Target Built upon a Benzodiazepine Antiviral

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Yellow Fever (YF) is an endemic disease in tropical areas and is caused by infection of Yellow Fever Virus (YFV), a mosquito-borne RNA virus that belongs to the genus Flavivirus. Besides the vaccine that has limited use after YF breakouts, anti-YF treatment is deficient and therapeutic development is urgently needed. Recently, a benzodiazepine acetic acid (BDAA) compound that inhibited YFV in cultured cells and infected hamsters was identified through a high-throughput screening. Genetic analysis of drug-resistant phenotype mapped the non-structural protein 4B (NS4B) as the target of BDAA, but the specific binding site has remained unclear. In this work, we designed BDAA-based clickable photoaffinity probes for investigating the detailed binding mode between NS4B and BDAA. Different photoreactive groups, including trifluoromethyl phenyl diazirine and aryl azide, were installed to the BDAA acyl sulfonamide bioisostere separately, with the corresponding photoaffinity probes presenting sub-micromolar EC50s, and the diazirine probe showing predicted and efficient photolysis patterns. Further modifications on BDAA revealed positions tolerated for alkyne incorporation. Attaching the photoreactive group and alkyne side chain at different moieties led to the discovery of BDAA-based clickable probes with promising antiviral activity in cellular assay, which indicated their potential for in-cell photo-crosslinking with NS4B and subsequent labeling site identification using pull-down assay and LC-MS/MS analysis.

081. Establishment of the First High-Throughput Screening Assay for Rhinovirus C Antiviral Drug Discovery

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Rhinoviruses (RVs) are the most prevalent cause of the common cold, but can also lead to more severe complications such as bronchitis and pneumonia in vulnerable populations. The RV-C species is of particular importance due to its contribution to exacerbations of asthma or chronic obstructive pulmonary disease impacting both infants and adults. The major hurdle in studying RV-C stems from the lack of a robust cell culture system supporting efficient virus replication. Here, we describe the development, optimization, and validation of the first cell-based high-throughput screening (HTS) assay for RV-C. This assay uses a newly engineered HeLa cell line overexpressing the RV-C receptor, cadherin-related family member 3, along with a GFP-expressing RV-C15. The improved permissiveness of this cell line allows efficient RV-C15 replication which yields higher viral titers and enables in-depth investigation into the life cycle of RV-C species. Our in vitro model also supports replication of another RV-C type (C41), further underscoring the ability of this model to support replication of the broad spectrum of RV-C types. Using RV-C15, we established an unprecedented high-throughput, high-content imaging-based antiviral assay for RV-C infection. By optimizing screening parameters, including cell seeding, infection conditions, liquid handling and image analysis, we achieved an assay quality with a Z'>0.75. Further miniaturizing the 96-well screening platform to 384-well format was successfully achieved and validated with known active antiviral compounds. Integration of this assay into HTS campaigns holds great promise for advancing antiviral drug discovery against RVs.

082. Suppression of hepatitis B virus replication and protein expression using CRISPR-Cas13b – pre-clinical investigations of a new antiviral approach

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New treatments targeting multiple stages of the hepatitis B virus (HBV) replication cycle are urgently required. The HBV RNAs represent a novel antiviral target, highlighted by recent siRNA and antisense oligonucleotide studies. Bacterial CRISPR-Cas13b endonuclease has been repurposed to target RNA in mammalian cells by designing highly specific 30 nucleotide guide RNAs (gRNAs) complementary to the target RNAs of interest. Here, in a world first study, we used CRISPR-Cas13b to target the HBV RNAs to reduce HBV replication and protein expression in vitro and in vivo. gRNAs were designed to target the HBV RNAs. HepG2 cells were transfected with wildtype (WT) HBV of multiple genotypes, Cas13b and gRNA plasmids. A HBV stable cell line and HBV infection model were transfected with Cas13b and gRNA plasmids. The impact on HBV replication and protein expression was determined. WT HBV, Cas13b and gRNA plasmids were hydrodynamically co-injected into CBA mice and sera hepatitis B surface antigen (HBsAg) was measured. Cas13b mRNA and gRNA were delivered by lipid nanoparticles (LNPs) in a HBsAg-expressing stable cell line and secreted HBsAg was measured. Cas13b strongly suppressed HBV replication and protein expression in all cell lines tested. The effect was pan-genotypic. Sera HBsAg was reduced by ~50% ($p<0.0001$) in vivo. LNP-encapsulated Cas13b mRNA reduced secreted HBsAg by 87% ($p=0.0168$) in a HBsAg-expressing stable cell line. CRISPR-Cas13b successfully targeted the HBV RNAs to significantly reduce HBV replication and protein expression in vitro and in vivo, demonstrating its potential as a novel antiviral for chronic HBV infection.

083. The CD8+T cells response is sufficient for protection with a CCHFV M-segment based DNA vaccine and GP38 enhances vaccine immunogenicity

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Crimean-Congo hemorrhagic fever virus (CCHFV) is the most widely distributed tick-borne virus of human medical importance. We developed a DNA vaccine expressing the full-length codon-optimized M-segment, which encodes the structural and non-structural viral glycoproteins. The vaccine, CCHFV-MAfg09, is highly immunogenic, eliciting both antigen-specific humoral and cellular immunity when delivered by intramuscular (IM) electroporation (EP). CCHFV-MAfg09 is completely protective against CCHFV-Afg09-2990 challenge in mice and significantly protective in non-human primates. We found that CCHFV-MAfg09 vaccinated mice lacking B-cells (μMT knockout mice) were still protected against lethal challenge with CCHFV, whereas mice lacking T-cells (TCRα/β knockout mice) and CD8+ T-cell deficient mice (CD8-/-) succumbed to disease. This data suggests that the CD8+ T-cells play a significant role in the protective response provided by the vaccine. Finally, we show that the non-structural M-segment protein, GP38, influences CCHF vaccine immunogenicity and provides significant protection from homologous CCHFV challenge. In contrast, the immune responses to vaccination with the structural glycoproteins alone were not sufficient for protection in mice. Our results demonstrate that M-segment DNA vaccines elicit protection primarily through the CD8+ T cell response and illustrates the immunorelevance of GP38 in mice. The significant efficacy of our M-segment based DNA vaccine makes it an ideal candidate to be combined with an efficacious nucleocapsid-based vaccine to produce a potentially broadly cross-protective vaccine effective against the multiple clades of CCHFV.

084. Unveiling host-cell glycosylation changes upon parainfluenza virus infection

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Human parainfluenza virus (HPIV) remains to be one of the major causes of respiratory illness particularly in young children, the elderly and immunocompromised people. There were 725, 000 hospitalizations and 34,400 deaths due to HPIV infections reported in 2018 alone globally. Despite the significant efforts in designing therapeutics, there is neither an effective antiviral nor a vaccine available against HPIV. Cellular glycosylations are known to play a pivotal role in HPIV biology. Glycans like sialic acid (Neu5Ac) have been identified as cellular receptors for HPIV and utilised as a molecular template for structure-based drug design. However, dynamics of the host-cell glycome upon HPIV infection has never been studied. Herein, we profile surface glycans (N-, O-, glycosphyngolipids) decorating the cell upon HPIV infection using state-of-the-art mass spectrometry techniques and instruments. We observed a significantly higher expression of oligomannose type N-glycans at the surface of HPIV-infected cells when compared to their mock-infected control, with correspondingly lower expression of complex type N-glycans. Unique O- and glycosphingolipids glycosylation features were also found on HPIV-infected cells. Based on these findings, we hypothesize that distinct glycan motifs on HPIV-infected cells may guide immune responses targeting the infected cells. Our next goal is to conduct a bulk RNA-Seq study to uncover the possible role of glycan changes on host-cell immune response. With all the results in hand, our study could pave the way to potentially identify novel target(s) for designing therapies to mitigate the impact of HPIV infection.

100. Ross River virus upregulates chemokine (c-c motif) ligand 5, enhancing metalloproteinase expression for virus-induced extracellular matrix degradation

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Ross River virus (RRV) causes crippling pain and joint inflammation that can be both acute and chronic. While it is well-established that RRV infection destroys the cartilage and synovial tissues, the underlying causes that contribute to this remain poorly defined. The extracellular matrix (ECM) is a significant component of cartilage and the joint and is essential for reducing friction and minimising shock in the joints caused by movement and everyday activity. Chondrocytes and fibroblast-like synoviocytes (FLS) play essential roles in extracellular matrix homeostasis by secreting ECM constituents and enzymes. Here, we conducted a gene expression analysis of RRV-infected FLS and observed increased levels of ECM breakdown enzymes such as ADAMTS4, ADAMTS5, MMP1, MMP3 and MMP13. Notably, the increase of MMP3 negatively modulates key ECM components including type II collagen, aggrecan and lubricin in chondrocytes suggesting an alteration in ECM through enzyme overexpression in FLS. Additionally, our extensive gene expression analysis with a PCR array of target genes known to play a role in arthritis identified significant increases in CCL5 expression in both RRV-infected FLS and chondrocytes. Most importantly, our preliminary data on CCL5 inhibition suggest that CCL5 may contribute to the increase of ECM breakdown enzymes.

Overall, our findings strongly support a model in which RRV modulates ECM integrity by overproducing ECM breakdown enzymes through the upregulation of CCL5 expression. This study provides new insight into the cellular and molecular mechanisms of virus-induced joint arthralgia and the potential use of CCL5 as a therapeutic target for antivirals.

101. Utilizing National Health Insurance Research Database in Taiwan Searches the Potential Traditional Chinese Medicines as Antiviral for Dengue Virus Infection

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Dengue fever is a formidable mosquito-borne viral disease with a potentially fatal trajectory, and now still lacks a dedicated antiviral drug despite its severity. Traditional Chinese Medicine (TCM) is well used in Taiwan as the prevalent treatment for various illnesses. This study capitalizes on the extensive Taiwan National Health Insurance Database (NHID), delving into TCM exposure patterns among Dengue Virus (DENV) and Dengue Hemorrhagic Fever (DHF) patients. To understand whether TCM might affect for treatment of dengue disease find out the possible antivirals. Validating their antiviral efficacy, an immunofluorescence assay (IFA) was used to assess the anti-DENV impact of selected TCM formulations. Sixty-two TCM formulas, incorporating frequently used options and promising targets, emerge as candidate choices. Preliminary findings spotlight at least 20 of these formulas for their potential to curtail the progression of dengue fever to a severe stage. Further assessing the cytotoxicity and anti-DENV effects of these 20 TCM formulas. Notably, Gastrodia elata (G. elata) and Pinellia ternate (P. ternate) revealed remarkable anti-DENV effects, particularly at multiplicities of infection (MOI) levels of 0.1 and 0.01. These promising outcomes underscore the potential antiviral properties of G. elata and P. ternate. In conclusion, this study introduces innovative methodologies utilizing the Taiwan National Health Insurance Database, shedding light on the antiviral prowess of G. elata and P. ternate, and paving the way for future research into effective antiviral interventions against dengue fever.

102. The Role of Fibronectin in the Pathogenesis of Mosquito-borne Viral Disease

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Ross River virus (RRV) and West Nile virus (WNV) cause mosquito-borne diseases that are nationally notifiable in Australia. More than 50% of RRV-infected patients develop joint pain that can persist for years while some WNV-infected patients develop neuroinvasive diseases with a high fatality rate. Despite decades of research, we still do not fully understand the pathomechanism of these diseases. Studies have shown that host factors play significant roles in infection outcomes, including in

the development of clinical symptoms. Therefore, we are currently investigating the involvement of certain key host factors in the pathogenesis of RRV and WNV. In our previous qPCR array findings, we saw an increase in the gene expression of fibronectin in chondrocytes following RRV infection. Fibronectin (FN1) is an extracellular matrix glycoprotein that promoted inflammation in many diseases such as rheumatoid arthritis, but its role in RRV and WNV infection and disease has not been studied. We have examined FN1 gene and protein expression in various cells which are known to be targeted by RRV and WNV during human infection. We found that fibronectin was decreased in RRV-infected synovial and fibroblast cells but increased in WNV-infected immune cells. Functional studies including protein overexpression or knockdown in vitro as well as in vivo studies in well-established mouse model will be performed to further dissect the role of fibronectin in disease pathogenesis. Knowledge from this study will be a vital stepping stone towards the development of novel therapeutics or vaccines which are currently limited for these viruses.

103. Old world alphaviruses induce rapid and strong neuroinflammation in primary human astrocytes

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Alphaviruses are a global health threat to humans and animals alike. These viruses cause acute and chronic illnesses, which can have detrimental sequelae and even lead to death. Typically, old-world alphaviruses are known to cause severe/debilitating arthritis. Yet, recently, neurovirulence has been consistently associated with chikungunya virus (CHIKV) infection. Astrocytes are the most abundant cell type in the brain parenchyma and are essential for maintaining homeostasis. They have many diverse and important functions such as supporting neuronal function by modulating the uptake and metabolism of neurotransmitters, gliotransmitters and ions. They regulate synaptogenesis, maintain the blood brain barrier, secrete neurotrophic factors, and transform oxygen free radicals into nontoxic species. Furthermore, they are key players in local immunity and inflammation through the production and secretion of cytokines, proteases, adhesion molecules, and extracellular matrix components. Herein, we explored the ability of old-world alphaviruses to infect primary human astrocytes. Using RT-qPCR and Bio-Plex® assays we show that when infected, critical innate immune responses such as IL-1β, TNF, IL-6, IFN-g, fractalkine and IL-10 are produced. Interestingly, the up-regulation of these cytokines is also seen in various dementias possibly explaining some of the sequalae seen in neuro-CHIKV cases. We further explored possible mechanisms driving alphaviral neuropathogenesis using NanoString nCounter neuroinflammation panel. Dissecting the molecular pathways involved in alphaviral induced neuroinflammation provides a foundation to identify potential novel therapeutic targets.

104. Identification of anisomycin as a novel inhibitor of Chikungunya virus

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Chikungunya virus (CHIKV) is an enveloped RNA virus of Alphavirus genus in the Togaviridae family. CHIKV causes Chikungunya fever (CHIKF), which is often characterized by fever, myalgia, and arthralgia. Although CHIKF was considered an endemic disease in tropical areas of Africa and Asia, it has been identified in more than 40 countries. Therefore, CHIKV infection is now considered as an increasing threat to public health worldwide. However, no specific drug is available for the treatment of CHIKV infection. In this study, we aimed to identify new antiviral agent(s) against CHIKV and to analyze their mechanism of antiviral inhibition. To search for chemical compounds that inhibit CHIKV infection, an In-Cell ELISA was performed using a CHIKV infectious clone and an alphavirus-specific antibody. Through a chemical library screen for autophagy-inducing or -inhibiting compounds, 7 compounds were found capable of reducing CHIKV infection in Vero cells by less than 20%

compared to DMSO-treated cells. Among the candidate inhibitors, anisomycin, a pyrrolidine-containing antibiotic isolated from Streptomyces griseolu, showed the highest inhibition of CHIKV replication without severe cytotoxicity. Anisomycin showed dose-dependent antiviral activity against two East/Central South African CHIKV strains, including an Indian Ocean Lineage, in Huh7 cells. A time-of-addition assay revealed that anisomycin exhibited anti-CHIKV effect at an early stage of viral replication. Although anisomycin is well established as an activator of MAPK signaling pathways, three major MAPK pathways (ERK, p38, and JNK) were unlikely to be involved in the anti-CHIKV activity of anisomycin. Further investigati

105. Unlocking the Flaviviral Blueprint: Leveraging Flaviviral Regulation of Selective Autophagy for Therapeutic Intervention

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There is an urgent need for new therapeutic development for flaviviruses such as dengue and Zika, due to the rapid global spread of its vectors, the complexity of flavivirus infections and the limited efficacy of current interventions. Elucidating the intricate host-virus interactions during establishment of infection could open avenues for novel therapeutic strategies. In this study we identified Ube2g2 as a host dependency factor for Zika virus (ZIKV) and characterized its role in fine-tuning autophagy via Ube2g2 to support viral replication and progeny assembly. CRISPR/Cas9-mediated knock-out of Ube2g2 dramatically reduced ZIKV production, which could be rescued by reconstituting the wild-type but not the catalytically deficient (C89K) mutant of Ube2g2, suggesting that its enzymatic activity is necessary. This deficiency resulted directly from a profound loss in replication organelle formation. We show that ZIKV utilizes Ube2g2's dual activity in (i) triggering lipophagy in conjunction with Aup1, and (ii) initiating degradation of ER chaperones to restrict ER-phagy upon Xbp1-IRE1 triggered ER expansion. Hereby we highlight how ZIKV orchestrates an intricate sequence of events required for efficient viral production, uncovering multiple stages at which therapeutic intervention could be developed. Moreover, Ube2g2 deficiency also resulted in a profound loss of infectious viral progeny in dengue virus and coronavirus infection, highlighting how host-directed therapeutics could have broad-spectrum potential, which is particularly relevant considering the challenges posed by co-circulation of flaviviral species.

106. Carbazole to indolazepinone scaffold morphing generates potent cell-active Dengue antivirals

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Dengue and other flaviviruses constitute unmet medical needs threatening >40% of the world population as there no antiviral medicines or safe vaccines are available. WHO has designated these viruses "serious threats" with increasing concern for outbreaks. For these reasons, developing treatments for dengue and Zika infections have been in the foreground of antiviral research.

The proposed oral presentation will describe our efforts in the scaffold morphing of our previous hits involving a series of transpositions of the three pharmacophoric domains in order to establish their best relative spatial arrangement. This was guided by the performance of the compounds in two cellular assays, namely plaque reduction and dose-dependent inhibition, and led to SP-1769B which exhibits excellent safety profile and much more favorable clogP in comparison with our previous hits. SP-1769B, a prodrug, is among the most cell-efficacious pan-serotype DENV inhibitors in the literature with EC50 of 100 nM in two serotypes and consistent performance in two different cell lines. SP-1769B also exhibits 100% inhibition of the secondary Dengue infection caused by ADE (Antibody-Dependent-Enhancement) due to cross-infection from different serotypes, a phenomenon that has hampered the development of dengue antivirals to date and rarely examined, if at all, in related works reported in the literature. We would also like to emphasize that the synthesis of all compounds mentioned was optimized thoroughly and we were able to prepare all derivatives in excellent quality in gram scale across 6-8 synthetic steps without need for chromatography.

107. Niosomal and poly lactic-co-glycolic acid nanoparticles loaded with cannabidiol as an antiviral strategy against Zika virus

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Zika virus (ZIKV) is transmitted mainly by the Aedes aegypti mosquito, and can cause neurological disorders and teratogenic effects; however, there are currently no specific vaccines or treatments for ZIKV infection. Cannabidiol (CBD), the primary non-psychoactive cannabinoid found in Cannabis sativa plant, has garnered significant attention due to its diverse effects on biological functions. Our recent study highlighted its antiviral activity against ZIKV and its impact on cell membranes. Nevertheless, it has been shown that CBD is photosensitive and the oral bioavailability is considerably low. To overcome these problems, we synthesized and characterized CBD loaded poly lactic-co-glycolic acid (PLGA) nanoparticles (NP) and niosomes (NIO). The PLGA-NP and the NIO were obtained using the phase inversion preparation and thin film methods, respectively. PLGA-NP smaller than 200 nm, while NIO particles ranged from 150 nm to values in the micro-scale were obtained. The results of MTT assay showed that none of the NP exhibited cytotoxicity. Regarding antiviral activity, PLGA-NP did not show significant inhibition against ZIKV. However, NIO presented significant inhibitions dependent on CBD concentration as it was an 86% inhibition at 100 µg/ml. Furthermore, vesicles containing different lipid and CBD molar ratios were prepared to study the effect of this cannabinoid on the formation and physicochemical properties of the NIO. The presence of CBD affected the size of the nanoparticles and decreased the Zeta potential in both nanosystems. In sum, the developed nanoformulations offer promising solutions for CBD stability, solubility, and its potential as an antiviral therapeutic.

108. Preclinical evaluation of insect-specific virus platform vaccines (ISVac) for Japanese encephalitis virus and Chikungunya virus in PC3 mouse models

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The World Health Organisation (WHO) recently launched the Global Arbovirus Initiative, highlighting the urgent need to address the increasing frequency and magnitude of arboviral disease outbreaks, which are primarily caused by mosquito-borne flaviviruses and alphaviruses. Japanese Encephalitis virus (JEV) and Chikungunya virus (CHIKV) are biosafety level 3 (PC3) arboviruses with significant health and economic consequences. Herein we present preclinical efficacy data of two new vaccine candidates which utilize Insect Specific Virus vaccine technology (ISVac). Binjari virus (BinJV) and Yada Yada virus (YYV) are an Australian insect specific flavivirus or alphavirus, respectively. Chimeric virus vaccines were created using the structural proteins of JEV or CHIKV, and the viral backbone of BinJV and YYV, respectively. These form authentic JEV or CHIKV virus-like particles when produced in insect cell culture, but cannot replicate in vertebrate vaccine recipients. Mice were vaccinated with BinJ-JEV or YYV-CHIKV via intramuscular injection (prime-boost schedule), which produced high levels of serum neutralizing antibodies against JEV or CHIKV. Upon challenge with JEV or CHIKV, mice were completely protected against viremia and disease. In addition, BinJ-JEV provided partial protection against viraemia and disease for the related Murray Valley encephalitis virus. In summary, BinJ-JEV and YYV-CHIKV effectively prevented viremia and disease in preclinical JEV and CHIKV challenge mouse models, and represent good candidates for further vaccine development.

109. Adjusting susceptibilities of C57BL/6 mice to flaviviruses for evaluation of antiviral drugs by altering the levels of interferon alpha/beta receptor function

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The purpose of this study was to optimize the infectivity of four different flaviviruses in mice for evaluating antiviral drugs by using 7-deaza-2′-C-methyladenosine as a prototypic small molecule. This was accomplished by using wild-type mice with intact interferon responses, type 1 interferon alpha/beta receptor knockout mice, or by injecting wild type C57BL/6 mice with varying doses of anti-type 1 interferon receptor antibody (MAR1-5A3) to optimize the infectivity and lethality of the four different emerging flaviviruses of this study: West Nile virus, Japanese encephalitis virus, deer tick virus (lineage 2 Powassan virus), and Usutu virus. The suitability of different mouse models depended on varied sensitivities of the viruses to the in vivo functionality of type 1 interferon activity. West Nile virus productively infected wild-type C57BL/6 mice to cause lethality, whereas Usutu virus required a complete absence of type 1 interferon receptor function. Deer tick virus and Japanese encephalitis viruses required a dampening of type 1 interferon responses by adjusting the doses of MAR1-5A3 antibody injections. Challenge dose-responsive mortality, weight loss, and viral titers were observed if the type 1 interferon responses were dampened with MAR1 5A3. Conversely, without MAR1-5A3 injections, these disease phenotypes were not viral challenge dose responsive. From these different interferon-responsive models, the appropriate lethality was identified to determine that 7-deaza-2′-C-methyladenosine has high efficacy for West Nile and Usutu viruses and low efficacy for deer tick and Japanese encephalitis viruses.

110. Development of messenger RNA vaccines targeting dengue virus non-structural proteins

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[Background] During the recent pandemic of COVID-19, the first-ever messenger (m) RNA vaccines were shown to be safe and highly effective in humans. This evoked us to develop safe mRNA vaccines that can prevent severe forms of dengue. Since an antibody-dependent enhancement (ADE) is the major obstacle for dengue virus (DENV) vaccine development, we have newly developed mRNA vaccines that target DENV non-structural (NS) proteins. Although these vaccines neither produce neutralizing antibodies nor prevent infection, they are expected to induce T cell immunity and prevent progression to severe dengue diseases without any concern for ADE.

[Methods] We have generated mRNA constructs that comprise DENV2 NS3 genome sequences. AG129 mice (deficient in IFN-α/β and γ receptors) or A129 mice (deficient in IFN-α/β receptor) were immunized with mRNA-Lipid nanoparticles (LNPs), followed by booster at 3 weeks, and lethally challenged with DENV2 at 6 weeks post-immunization.

[Results and Conclusion] T cell responses specific to NS3 could be induced in both AG129 and A129 mice immunized with NS3 mRNA at a similar level. Interestingly, however, immunization with NS3 mRNA in AG129 mice did not show efficacy on either mouse survival rate or viremia reduction. Significantly, on the other hand, immunization with NS3 mRNAs in A129 mice resulted in 100% mouse survival and significant levels of viremia reduction, suggesting an important role of IFN-γ signaling in T-cell function. Thus, mRNA vaccines targeting DENV NS proteins would be promising vaccine candidates that can abolish any concern for ADE and the use of A129 mice would provide significant advantages in the vaccine development.

111. Serotype-Specific Regulation of Dengue Virus NS5 Protein Subcellular Localization

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Dengue virus (DENV) non-structural protein 5 (NS5), consisting of methyltransferase and RNA-dependent RNA polymerase (RdRp) domains, is critical for viral RNA synthesis within endoplasmic reticulum-derived replication complexes in the cytoplasm. However, a significant proportion of NS5 is known to be localized to the nucleus of infected cells in the case of DENV2, 3 and 4, whereas DENV1 NS5 is predominantly cytoplasmic. We still have an incomplete understanding of how DENV NS5 nuclear localization is regulated. Within NS5, 2 putative nuclear localization signal (NLS) sequences have been identified; NLSCentral, residing in the palm of the RdRp domain, as well as the recently discovered NLSC-term residing in the flexible region at the C-terminal of the RdRp domain. We have previously shown that DENV2 NS5 nuclear localization can be significantly reduced by single point mutations to the NLSC-term. Here, we present biochemical, virological and structural data demonstrating that the relative importance of either NLS in NS5 nuclear localization is unique to each of the 4 DENV serotypes; DENV1 NS5 subcellular localization may occur through a weak interaction between importin-alpha (IMPα) and NLSCentral, DENV2 NS5 is almost exclusively nuclear through strong interaction between IMP and NLSC-term, while both NLSs of DENV3 NS5 contribute to directing its nuclear localization. Lastly, in the case of DENV4, NS5 nuclear localization appears to be associated with the C-terminal region and dependent on either a post-translational modification or an IMPα-independent pathway.

115V. A Yellow Fever Virus NS4B Inhibitor sequentially Inhibits Viral RNA Synthesis and Activates Double-Stranded RNA Reponses to Accelerate Apoptosis of Infected Cells

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Yellow fever virus (YFV) RNA replication takes place in the membranous vesicle compartments derived from the invagination of endoplasmic reticulum membranes, designated as replication organelles (ROs). Non-structural protein 4B (NS4B) of flaviviruses play essential roles in the biogenesis of replication organelles (ROs) and evasion of innate immune responses. We reported previously that YFV NS4B inhibitor acetic acid benzodiazepine (BDAA) induced the leakage of viral double stranded RNA (dsRNA) from ROs and activation of proinflammatory cytokine responses in infected cells. Further mechanistic studies showed that BDAA treatment of YFV-infected cells promptly inhibited YFV RNA synthesis in 15 to 30 min and activated cytoplasmic dsRNA sensors, including protein kinase RNA-activated (PKR), RIG-I-like receptors (RLRs) and 2 ,5 -oligoadenylate synthetases (OASes), in 30 to 90 min and apoptosis of infected cells in 3 to 6 h. Gene knockout analysis showed that while RLR pathway was primarily responsible for YFV infection induced and BDAA enhanced inflammatory gene response, all the three RNA sensor pathways contributed to YFV induction of apoptosis. However, RLR and RNase L pathways were predominantly responsible for BDAA enhancement of YFV-infected cell apoptosis. Interestingly, although triple knockout of RLR-adaptor protein MAVS, PKR and RNase L completely abolished YFV infection-induced and BDAA enhanced cytokine response and apoptosis, it only very modestly attenuated the antiviral activity of BDAA. Our findings not only uncover the unique mode of action of BDAA, but also shed new light on the roles and mechanisms of NS4B in YFV RNA synthesis and immunopathogenesis.

200. Meta-analysis of clinical and virological data in the Syrian hamster model of Nipah virus disease to support translation to human disease and utility for antiviral screening

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Nipah virus (NiV) causes severe respiratory and neurological disease in humans, with fatality rates reaching up to ~70%. Syrian hamsters are advantageous for modeling the full clinical spectrum of NiV infection reported in humans, but model development and associated natural history studies are complex and necessitate relatively large sample sizes. Here, using a sizeable historical data set representing 14 independent NiV hamster studies conducted at the CDC (Atlanta, USA) over the past 5 years (>200

hamsters), we analyzed clinical parameters including survival, daily weights, clinical scores and body temperatures, and viral tissue loads. Using the power offered by the large sample size, we investigated experimental design-associated differences (virus strain and dose) to critically inform design of medical countermeasure studies and to improve interpretation of resultant data. We found that outcome of infection was associated with viral strain and route of infection: lethality was higher when strain Malaysia was administered via the intraperitoneal (IP) route, and strain Bangladesh by the intranasal (IN) route. The full spectrum of disease (respiratory and/or neurological signs) was typically seen with all infection routes and strains, yet strain-associated predominance for respiratory or neurological signs was consistent with human epidemiologic data. Overall, we present a unique collective analysis of clinical parameters and tissue samples from the hamster model of disease and provide a core foundation of data to support the utility of this model for NiV antiviral and vaccine development efforts.

201. Discovery of Small Molecule Inhibitors against Henipaviruses

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It has been more than two decades since the initial outbreaks of respiratory disease and encephalitis caused by the highly pathogenic henipaviruses Hendra virus (HeV) and Nipah virus (NiV) and yet there are no approved antiviral therapeutics or vaccines available for human use. The isolation of Cedar virus (CedV), a nonpathogenic henipavirus closely related to HeV and NiV provides a unique opportunity to investigate not only differences in pathogenesis between these viruses but also its use as novel reporter viruses and as a potential vaccine platform. We recently established a cell-based high throughput screening (HTS) assay using a recombinant CedV expressing luciferase (rCedV-Luc), from which we identified new compounds that effectively inhibited authentic NiV-Bangladesh (NiV-B) and HeV replication. Here, using the rCedV-Luc HTS assay we have further optimized the inhibitory activities of the validated compounds by structure activity relationship (SAR) development. We evaluated the efficacy of ~80 derivatives and identified new compounds that inhibited rCedV-Luc with IC50 values < 1 μM with minimal cytotoxici

202. Antiviral activity of pyrophosphate analogues against arenavirus infection

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Junín virus (JUNV) is an arenavirus that can cause acute hemorrhagic fever syndrome, a non-eradicable endemic zoonotic disease in Argentina and for which there is no specific antiviral treatment. It is known that arenaviruses associate with cholesterol-enriched membrane microdomains for their budding and assembly. This evidence supports the importance of further studying lipid metabolism during JUNV infection. The enzyme farnesyl pyrophosphate synthetase (FPPS) is a key enzyme in the mevalonate metabolic pathway. In this pathway, cholesterol and other lipids that are part of the cell plasma membrane, including lipid rafts, are synthesized. Phosphate and pyrophosphate analogs directly inhibit the enzymatic function of FPPS. These molecules have been used for more than four decades as therapy to treat diseases associated with calcium reabsorption, and various types pf cancer. These analogues emulate the substrates involved in the mevalonate pathway, acting directly on FPPS. Therefore, the goal of this work was to evaluate the antiviral activity of pyrophosphates analogues against infection with Junín arenavirus. For this, a screening of 8 chemically synthesized compounds and 1 commercial compound was performed in Vero cells, for cell viability and antiviral activity. Compounds BP 9, BP 13, BP 17 and BP 18 showed promising results. To note, the commercial compound zoledronic acid (ZA) presented lower antiviral activity. The antiviral effect was studied varying the time of exposure to the treatment with intervals of 6 to 5 hours post-infection, observing 1 log difference in viral yield at late time-point treatments. It worth to mention that no virucidal activity was detected.

203. N-substituted Pyrrole-based Heterocycles as Broad-spectrum Filoviral Entry Inhibitors

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Ebola (EBOV) and Marburg (MARV) viruses are priority infectious agents due to their aggressive disease state and high lethality in infected patients. The current FDA-approved antibody treatments are only specific to Ebola Zaire, which excludes the other EBOV species and MARV; thus, the need for novel therapeutics effective against diverse filoviruses remains. The filoviral glycoprotein (GP) has proven to be a drug-targetable site as it is conserved among all filoviruses and is used to mediate numerous steps in filoviral entry. In our effort to develop broad-spectrum antifilovirals, we have discovered a series of N-substituted pyrrole-based heterocycles that target GP and effectively inhibit diverse filoviral entry via an HIV-based pseudovirus assay. Selectivity and potency of these viral entry inhibitors were improved by introducing structural modifications to the heterocyclic core, N-substituents, and the amide-amino linker. The lead inhibitor displayed sub-micromolar EC50 values, 4.3-fold potency improvements compared to the hit, and a selectivity index greater than 100. In addition, antiviral activity was validated using replication-competent EBOV and MARV, mutational analysis was used to identify the suggested EBOV GP binding region, and antiviral counter-screen and phospholipidosis assays demonstrated reduced off-target activity for these filoviral entry inhibitors. Excellent activity coupled with favorable drug-like properties exemplifies these N-substituted heterocycles as great candidates for further pursuit in the advancement of novel broad-spectrum antifilovirals.

204. Design, synthesis, and biological evaluation of β-L-thymidine, β-L-2'-deoxycytidine, and other L-nucleoside reverse fleximer analogues

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Both β-L-thymidine (LdT) and β-L-2'-deoxycytidine (LdC) have potent anti-HBV activity, with LdT later becoming the pharmaceutical drug Telbivudine. However, like all current anti-HBV drugs, LdT is unable to fully clear HBV from the body and is susceptible to resistance caused by common HBV point mutations. Additionally, co-infections with other viruses is not uncommon, including Ebola and Dengue. Other viruses, such as Epstein-Barr Virus (EBV), have been linked to causing HBV reactivation.

Fleximers are a shape-modified purine nucleoside analogues with a split ring system, endowing the molecule with increased flexibility. They have been shown to have increased, broad-spectrum activity compared to their parent compounds and proven to be able to overcome point mutations. Similarly, reverse fleximers have the connectivity reversed where the glycosidic bond is to the 6-membered ring. By applying this technology to nucleosides like LdT and LdC, there is the potential to impart broad spectrum antiviral activity as well as decrease susceptibility to point mutations.

Two reverse fleximer analogue series were synthesized, with parent compounds LdT and LdC. Additional series are also being worked on, including β-L-ribose and another based on the HBV drug lamivudine. Initial data has shown several LdC analogues with broad-spectrum antiviral activity as well as one LdT analogue with activity against Epstein-Barr virus which has been associated with HBV reactivation. Additionally, some anti-SARS-CoV-2 activity was observed with a β-L-uridine reverse fleximer analogue. Further optimization is currently underway with a preliminary SAR study and exploration of prodrugs.

205. Identification and characterization of small molecule inhibitors of SARS-CoV-2 RNA dependent RNA polymerase by targeting the NSP8-NSP12 interface

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COVID19 stands as one of the most significant public health concerns in recent human history. Mutations of SARS-CoV-2 lead to the generation of new variants with the potential to cause varying degrees of morbidity and mortality. Therefore, it is imperative to identify and develop effective therapeutics that target virus specific conserved protein. The RNA-dependent RNA polymerase (RdRp) plays a pivotal role as a major component of the viral replication-transcription complex (RTC) and is essential for virus replication. Furthermore, RdRp lacks a human homologue and is highly conserved, making it an attractive target for therapeutic development. In this study, we expressed three proteins of the RTC, namely nsp12, nsp8 and nsp7, and assessed their ability to form a minimal RTC capable of transcribing an RNA molecule. Additionally, we developed a nanoluciferase based complementation assay to investigate the interaction between nsp12 and nsp8, utilizing nsp12-NanoNp52 and nsp8pep-NanoCp52 as interacting partners. The complementation assay was subsequently employed to screen small molecule libraries to identify candidate compounds that inhibit RdRp activity. We successfully identified several molecules that inhibited the RTC activity. These candidate inhibitors were further characterized biochemically and demonstrated binding affinity to nsp12. Our cell-based assay revealed the antiviral activity of these compounds against the Washington strain of SARS-CoV-2, with EC50 values in low micromolar range. Further investigation is necessary to fully characterize these small molecules and evaluate their efficacy in animal models.

206. Metformin Inhibits Replication of EV-A71 and CVA16 by Multiple Mechanisms

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Hand, foot, and mouth disease (HFMD) is a global health problem that often affects children under the age of 5. HFMD, caused by enterovirus A71 (EV-A71) and coxsackievirus A16 (CVA16) infections, is usually self-limited, but occasionally can lead to severe neurological complications, pulmonary edema, and even death. Unfortunately, there are currently no effective drugs available for the prevention and treatment of HFMD in clinical practice. Therefore, the development of anti-HFMD drugs is urgently needed. Drug repurposing is an effective strategy for treating viral diseases. Here, we found that the classical antidiabetic drug metformin inhibits the replication of EV-A71 and CVA16 in vitro. Mechanistically, metformin was not directly viricidal. Instead, metformin can inhibit the replication of EV-A71 and CVA16 by inhibiting the transcription of cellular pseudokinase tribbles 3 (TRIB3) to down-regulate the protein level of scavenger receptor class B member 2 (SCARB2), which is a receptor for EV-A71 and CVA16. In addition, metformin inhibits the production of inflammatory cytokines induced by viral infection via modulating nuclear factor kappa B/mitogen-activated protein kinases (NF-kB/MAPK) pathway, thereby inhibiting the replication of EV-A71 and CVA16. These findings suggest that metformin could be a potential anti-HFMD drug in the future.

207. Defective interfering RNA induces innate immunity and broad-spectrum antiviral activity

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RNA viruses like dengue (DENV), influenza A (IAV), respiratory syncytial virus (RSV) and SARS-CoV-2 (CoV-2) pose challenges to global health systems due to their phenotypic plasticity, evading immunity and antivirals. The rapid evolution of human viruses and the potential threat from unknown viruses reinforces the need for new broadly acting antiviral medicines. The retinoic acid-inducible gene I (RIG-I) is an essential cytosolic pattern recognition receptor (PRR) that recognises viral RNA and activates interferons (IFNs) and cytokines in response to viral infections. RIG-I agonists have great potential as antivirals for treating viral pandemics. Our project focuses on designing and applying an efficient delivery system for the novel RIG-I agonist, DI290 RNA. DI290 RNA is 290 nucleotides long and was identified through in vivo and in vitro screenings of defective interfering (DI) RNAs made by DENV. Our results demonstrate the efficacy of DI290 RNA in activating RIG-I-mediated type I IFN response and suggest that DI290 RNA can be used as a novel, effective RIG-I agonist that inhibits viral infections. We also studied the ability of DI290 RNA to provide pan-antiviral protection and defence against different viral infections. To achieve this, we utilised robust tissue culture and synthetic systems to produce different types of nanoparticles containing DI290 RNA. Our experiments show that DI290 has broad-spectrum activity, capable of inhibiting the replication of DENV, RSV, yellow fever virus, zika virus, Japanese encephalitis virus and CoV-2. In vitro and in vivo studies will be presented.

208. Plitidepsin broadly inhibits protein synthesis of distant viruses while reprograming the translational cellular landscape as a homeostatic response

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Plitidepsin is an antitumoral drug safe for treating COVID-19 infection that impairs SARS-CoV-2 replication by targeting the translation elongation factor eEF1A. Here we used deep quantitative proteomics and functional infectious or mRNA reporter assays to delineate the antiviral cellular landscape induced by plitidepsin. Active doses of plitidepsin inhibited the replication of

SARS-CoV-2 Omicron lineages. Early translation of viral R1AB polyproteins was reduced along the late synthesis of structural proteins such as the nucleocapsid. Plitidepsin decreased translation of viral and non-viral positive sense RNAs, inhibiting de novo protein synthesis without perturbing cellular viability. Less than 14% of the cellular proteome was affected by plitidepsin, which despite being a protein synthesis inhibitor, up-regulated interactors of eEF1A key for switching to cap-independent translational routes (such as those mediated by eIF4G2, YTHDF1, PABP1, IF2B2, and ribosomal proteins). Plitidepsin thus favored proteostasis via alternative protein synthesis pathways, but also augmented inhibitors of canonical protein translation such as EIF2AK3, ZFP36L1 or AGO2. This led us to test its activity against other unrelated viruses, where plitidepsin inhibited the replication of members from the Flaviviridae, Pneumoviridae and Herpesviridae families. Yet, it failed to block retroviral proviruses that can exploit cap-independent translation pathways reprogrammed by plitidepsin. By unraveling the cellular antiviral landscape elicited by plitidepsin we identified a novel broad-spectrum antiviral with potential to counteract future pandemic viruses.

209. Broad-spectrum antiviral potential of PI3K inhibitors

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The phosphatidylinositol-3-kinase (PI3K) pathway is central to many cellular functions like growth, survival, metabolism, and the immune response. Persistent activation of the PI3K pathway has been linked to cancer, and some PI3K inhibitors are clinically approved against cancer.

Viruses have co-evolved to hijack many host cellular pathways including PI3K to support their lifecycle and evade immune clearance. Some PI3K inhibitors have published antiviral activities against several viruses including SARS-CoV-2. We examined the potential to repurpose a selection of PI3K inhibitors already in various stages of development against cancer to reveal antiviral activities. Eight PI3K inhibitors were examined in vitro for antiviral efficacy against human betacoronavirus OC43, four variants of SARS-CoV-2, influenza A virus and herpes simplex virus using infectivity assays. Compound toxicity profiles were measured using a metabolic assay. Two of the eight compounds demonstrated low micromolar potency against all the viruses examined, demonstrating broad-spectrum antiviral activities across the three unrelated viral families. When examined in combinational studies with clinically approved SARS-CoV-2 antivirals Remdesivir and Nirmatrelvir (Paxlovid) against the Omicron variant, one compound showed strong drug synergy with both approved antivirals, allowing significant dose reductions to achieve the same antiviral effects, which reduces risks of side effects and raises barriers to resistance, while pharmacological data showed tolerability. Our study demonstrates therapeutic potential in repurposing PI3K inhibitors as broad-spectrum antiviral candidates for further development.

210. Evaluation of Potency and Metabolic Stability of Diphyllin-Derived Vacuolar-ATPase Inhibitors

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Diphyllin, a natural product, exhibits a diverse array of biological activities, including broad-spectrum antiviral activity. The utility of diphyllin and its natural and synthetic derivatives have been noted for Ebola, Zika, Influenza, and SARS-CoV-2, among other viruses. The primary target for the antiviral activity is derived from diphyllin's inhibition of the host factor vacuolar-ATPase

(V-ATPase). V-ATPase is responsible for acidifying endosomal compartments, playing a key role in lysosomal trafficking. In the context of viral entry, this acidification initiates a cascade of events, ultimately allowing the virus to fuse to the endosomal membrane and release its genome into the cytoplasm. To mitigate transmission due to bodily fluids and reduce necessity for hospital-administered injectable therapeutics, we focused our efforts on developing orally bioavailable lead candidates. The structure-activity, metabolism, and pharmacokinetics of diphyllin derivatives have been studied, despite in vivo use with several disease models. Diphyllin and most of its derivatives lack suitable potency and metabolic stability to be antiviral drug candidates. We have synthesized a series of novel derivatives and scaffold analogs, resulting in leads with improved antiviral potency, oral bioavailability, and pharmacokinetic profiles. These additionally prove useful for defining the receptor binding site in a specific subunit of V-ATPase. Overall, these studies indicate the potential for design of a variety of V-ATPase inhibitors with suitable properties to target a broad spectrum of viral pathogens.

211. Curcumin affects early and late steps of Junin virus multiplication in cell cultures

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The arenavirus Junin (JUNV) is the causative agent of the Argentine haemorrhagic fever (AHF), a severe zoonotic disease of the central area of Argentina. Although AHF is preventable by vaccination, antiviral therapy deserves further investigation due to contraindications to the use of an attenuated vaccine. Curcumin (CUR) is a polyphenolic compound present in the root of Curcuma longa. Antiviral activity of CUR has been described for several viruses. In the present work, the in vitro antiviral effect of CUR against JUNV was studied. CC50 of the drug was determined in Vero cell monolayers treated with the compound for 48 h by MTT method. IC50 of CUR was determined by a yield assay of infected cells under different conditions of treatment. When cell cultures were pretreated with CUR, a SI of 372 was obtained. Both early stages of viral multiplication, absorption and internalization, were partially blocked by 80 µM CUR. The latter was associated to the destabilisation of the actin cytoskeleton by CUR. CUR also reduced the expression of viral nucleoprotein (N) and glycoproteins (G1 and G2) by WB and IFA. When a late stage of multiplication was investigated, the formation of syncytium was impaired although the pattern of G1 in the membrane was not affected. This observation leads us to speculate about the participation of CUR in the inhibition of a late stage of infection. This was confirmed by the fact that when the compound was added at 8 and 12 h p.i., viral yield was markedly reduced. These results show that curcumin inhibits Junin virus multiplication in cell cultures, targeting several stages of the viral cycle suggesting a multi-step inhibition.

212. Targeting SARS-CoV-2 Nsp14 Methyltransferase: From In Silico Design to Nanomolar Inhibitors

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The COVID-19 pandemic, caused by SARS-CoV-2, spurred the development of advanced antiviral strategies. Coronaviral methyltransferases nsp14 and nsp16, crucial for RNA capping, have emerged as potential drug targets, which could be inhibited using small molecules. Inspired by the endogenous inhibitor – S-adenosyl-L-homocysteine (SAH), we have designed bisubstrate inhibitors and assessed their inhibitory potential using in-silico docking and scoring. The most promising compounds were subsequently synthetized and tested in an enzymatic assay. Notably, some of these molecules exhibited nanomolar inhibitory activity against nsp14, showcasing their efficacy in disrupting crucial aspects of viral life cycle. Our inhibitors interact with amino acid residue F426, which is highly conserved across coronaviruses, suggesting broad-spectrum anti-coronaviral potential. Additionally, our compounds exhibit excellent metabolic and plasma stability, enhancing their developmental prospects. Ongoing research includes assessing their antiviral activity in cell cultures, which is a crucial marker of practical applicability.

In summary, we are addressing the pressing need for effective antiviral solutions in the face of current global health crises by discovering and developing a promising class of potent nsp14 inhibitors.

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213. Antiviral activities of two nucleos(t)ide analogs against orthopoxviruses

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Orthopoxviruses are a group of viruses that can cause diseases in both humans and animals. Some of the best known orthopoxviruses include the ones that cause smallpox and mpox (formerly monkeypox). Developing novel drugs to target poxviruses is needed, and identifying novel antiviral compounds is an important step in this process. We previously screened over 3,000 compounds ad identified multiple antiviral hits against orthopoxviruses. In this study, we characterized two hits, nucleoside trifluridine and nucleotide adefovir dipivoxil, for their antiviral activities against vaccinia virus (VACV), mpox virus (MPXV), and cowpox virus (CPXV) in primary human fibroblasts using Gaussia luciferase-based reporter assay and plaque assay. The results showed that both hits were effective in inhibiting the replication of VACV, CPXV, and MPXV. In addition, both inhibited VACV DNA replication and downstream viral gene expression. These findings suggest that trifluridine and adefovir dipivoxil are promising candidates for further development as effective drugs against poxviruses, including mpox.

215. The Extracellular Matrix Multi-Tissue Platform (MTP), Has Broad-Spectrum Antiviral Activity and Prevents Varicella Zoster Virus Spread in Cells and Human Skin

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MTP is a blend of extracellular matrix components from animal tissues, mainly collagen, elastin, and fibronectin. The powdered form is approved for human use as a dressing and treatment for wounds and burns. The gel form was found to broadly inactivate virions of Duck Hepatitis B virus, Coronavirus OC43, Herpes Simplex Virus 1, Human Immunodeficiency virus 1, West Nile Virus, Rubella virus, and Influenza B virus. It was less active against virions of Rabies virus, Vaccinia virus, Influenza A virus H1N1 and H3N2. We evaluated MTP against Varicella Zoster virus (VZV), which causes chickenpox and herpes zoster (shingles), in cells and human skin organ culture. MTP was effective against VZV as a pre-formulated gel (EC50 1.1%, CC50 28%, SI 25) and gel extract diluted in tissue culture medium (EC50 4.5%, CC50 75.9%, SI 17). Time-of-addition studies showed that MTP gel (12.5%) and gel extract (25%) inactivated cell-free VZV, blocked attachment and entry, and inhibited cell-cell spread when added up to 24 h post-infection. In human skin culture, MTP gel (undiluted) and powder prevented virus spread, even when treatment was delayed up to 5 days post-infection. Histopathological analysis showed that MTP was not toxic to the skin. Studies in NuSkin mice are underway to evaluate MTP gel and gel extract against VZV in vivo. Overall, the Multi-Tissue Platform has the potential to reduce the infectivity of a wide range of viruses, especially in the skin lesions caused by VZV or herpes simplex viruses. There is a need for topical treatments that speed lesion healing and reduce virus spread. This work was supported by NIAID DMID contract HHSN272201700030I.

216. Therapeutic efficacy of EIDD-2947 against poliovirus and coxsackievirus B3 in mice

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EIDD-2947 (EIDD), a lipophilic prodrug of EIDD-2749 (4'-FlU), was evaluated against the poliovirus (PV) and coxsackievirus B3 (CVB3) in PVR-IFNAR-/- and A/J mouse models, respectively. For both PV and CVB3 therapeutic studies, male and female mice were administered by oral gavage with 18 mg/kg/day initiated at 6, 24, and 48 hours after the viral challenge and continued for 10 days. The dosages and designs were based on the maximum tolerated dose and prophylactic studies in their respective models. Survival and viremia were the primary readouts. From the PV therapeutic study, EIDD-2947 statistically improved survival at all the tested time points ($p < 0.0001$) and statistically reduced the serum and tissue titer at 3 and 4 dpi, respectively. For the CVB3 study, EIDD-2947 improved survival when treatment was delayed: 6 hours ($p < 0.0001$), 24 hours ($p = 0.0040$), and 48 hours ($p = 0.0391$). Infectious viral titers were not statistically reduced at the peak of viremia at day 2 when treatment was delayed at 6 and 24 hrs. Histopathological evaluation revealed myocardial degeneration in heart tissues of the placebo-treated group, but no significant lesions were observed when treatment was delayed 6 hours compared to 24- and 48-hours. CVB3 infection caused tachycardia in infected vehicle-treated mice, but heart rate improved at 6 and 8 days post-infection when treatment was delayed up to 6 hours after the viral challenge. These studies reveal an important pre-clinical feature: the ability to treat up to 48 hours after viral exposure and that EIDD-2947 may have broad-spectrum antiviral efficacy in the evaluated enterovirus models.

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217. GS-7682, a Novel Prodrug of a 4′-CN-4-Aza-7,9-Dideazaadenosine C Nucleoside with Broad-Spectrum Activity and Efficacy in RSV-infected African Green Monkeys

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Acute respiratory viral infections (ARVI) are a major cause of global morbidity and mortality. Individuals with underlying chronic obstructive pulmonary disease (COPD) and asthma are particularly vulnerable to ARVI due to the risk of exacerbation of disease caused by these viral infections. A research program targeting respiratory syncytial virus (RSV) led to the discovery of GS-7682, a novel phosphoramidate prodrug of a 4 -CN-4-aza-7,9-dideazaadenosine C nucleoside (GS-646089) which is capable of metabolizing to high levels of the active triphosphate (GS-646939) in lung cells. GS-7682 has potent in vitro activity against multiple viruses in the pneumovirus (RSV and human metapneumovirus (hMPV)) and picornavirus (rhinovirus and enterovirus) families with EC50 values ranging from 46-210 nM and 54-90 nM respectively. Oral delivery of phosphoramidate nucleotide prodrugs frequently results in low exposure of the intact prodrug to the lung due to high first-pass hepatic extraction. However, direct delivery of GS-7682 to the respiratory tract via intratracheal administration resulted in high lung levels of the active triphosphate, which was associated with strong in vivo efficacy in an African green monkey model of RSV infection manifested by 3.6 log10 reduction in RSV viral RNA recovered from bronchoalveolar lavage fluid (BALF) at day 5 post-infection. These findings support additional evaluation of GS-7682 and its analogs as a potential therapeutic for ARVIs.

218. Novel Virucidal Synthetic Polymers Targeting Enveloped Viruses

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Introduction: 2020 demonstrated the world's inadequate preparedness for global viral pandemics. It is impossible to predict the next pandemic and the likelihood of coronavirus or influenza pandemic is high. Thus, urgent development and validation of effective antivirals is crucial for immediate stockpiling. Here, we described broad-spectrum virucidal synthetic polymers against enveloped viruses; influenza A virus (IAV) and SARS-CoV-2.

Methods: Utilizing oxygen-tolerant photopolymerization technique, polymers could be synthesized in a high throughput manner in 96-well plates, allowing direct translation into antiviral testing. Biocompatible, hydrophobic, and charged monomers were polymerized to identify structure-activity relationships. Antiviral activity was assessed by immuno-plaque assay in MDCK cells for IAV and Vero E6 cells for SARS-CoV-2. Polymer cytotoxicity was evaluated using the ATPlite 1-step luminescence assay.

Results: Screening 468 synthetic polymers identified 21 hits providing over 85% protection at 100 µg/ml (with almost similar activity at 50 µg/ml) against IAV, with seven emerging as lead compounds exhibiting non-toxicity (>85% cell viability). These polymers demonstrated consistent anti-IAV virucidal activity, regardless of dilution, with IC50 values ranging from 0.31 to 37.9 µg/ml. Additionally, six polymers demonstrated over 80% protection against the SARS-CoV-2 omicron variant. The polymers also showed promising post exposure anti-IAV activity.

Conclusion: Our findings suggest that the combination of high-throughput polymer synthesis and in vitro antiviral screening is a novel approach to identify broad-spectrum virucidal synthetic polymers.

219. Nasodine (0.5% PVP-I) Reduces SARS-CoV-2 Titers in COVID-19 Patients: a Phase II Trial

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Methods and Results: A multicenter, randomized, double blinded, placebo-controlled Phase II clinical trial to determine if treatment with Nasodine (GMP manufactured 0.5% povidone-iodine nasal spray) may be a useful adjunct in the management of COVID-19 and to evaluate its potential role in future respiratory virus outbreaks. Adult subjects with early COVID-19 symptoms were enrolled during the 2022/23 South African outbreak. Subjects received either Nasodine or placebo 8 times daily over 3 calendar days. SARS-CoV-2 titers were evaluated via daily swabs collected pre-treatment through day 5. Nasodine subjects exhibited significantly improved reduction in nasal viral load (log10 TCID50) on Days 2-4 compared to Placebo recipients (p=0.028), despite substantially lower recruitment numbers compared to the target (23 in the ITTi compared to a target of 144). Subjects exhibited improved rate of clearance of viable virus (p=0.032): viable virus was cleared (reduced to undetectable) from 70% of Nasodine recipients by day 3 (compared to 46% of placebo recipients) and 100% by day 4 and remained undetectable in both nasal and throat samples at day 5.

Conclusion: 20 doses of Nasodine® administered over 2.5 days significantly reduced the titers of viable SARS-CoV-2 virus in the nasal passages of COVID-19 subjects over days 2-4 compared to placebo (p=0.028). Nasodine may be useful in reducing viral titers and transmission risk during COVID-19 outbreaks and as a readily deployable, broad-spectrum, well tolerated, and cost-effective intervention for other outbreaks of upper respiratory tract diseases.

Registration: SANCTR: DOH-27-032022-7241 (29 Mar 2022)

220. Development of an antiviral assay in a specialized 3D liver tissue model in normal human-derived liver epithelial cells (EpiLiver™ LIV-100)

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In vitro assays are a critical step to determine the antiviral activity of novel compounds. Antiviral efficacy of compounds is often evaluated in immortalized cell lines which are cost effective, easy to use, and reproducible. However, these monolayer cell lines are not always predictive for cytotoxicity or in vivo efficacy. The EpiLiver, human primary cell-based 3D organotypic liver/hepatocyte tissue model consisting of normal, human-derived liver epithelial cells provides a human-relevant tissue model for human hemorrhagic fever viruses and echovirus antiviral research. We developed an antiviral assay to evaluate promising compounds against Rift Valley Fever Virus (RVFV), Tacaribe Virus (TCRV), Dengue Virus type 2 (DENV-2), Yellow Fever Virus (YFV) and Echoviruses 11 and 30 (Echo11 & 30). This assay has been used to evaluate virus replication, compound cytotoxicity, and antiviral efficacy within the EpiLiver™ model. This model could be used to evaluate other hepatotropic viruses. We show that ribavirin, favipiravir, NITD-008, EIDD-1931 and enviroxime significantly reduce viral replication of RVFV, TCRV, YFV, DENV-2 and human echoviruses, Echo11 and 30 as evidenced by 90% effective concentrations (EC90s) in the low µg/ml range. Additionally, the 3D liver tissue model is more sensitive for detecting antiviral efficacy as EC90 values were approximately 2- to 20-fold lower in 3D liver tissue compared to immortalized cell lines. The EpiLiver™ human tissue model is a valuable tool for evaluating antiviral compounds prior to advancement to in vivo models for acute drug permeation, safety, and antiviral efficacy studies against additional viruses which may induce liver damage.

221. Modifying Potent Broad-Spectrum Antiviral Imino-C-Nucleosides to Yield New Antiviral Leads

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Broad-spectrum antiviral nucleosides are an important class of molecules with proven potential to treat viral diseases.1 Both RNA and DNA viruses are susceptible to these modified nucleotides that interfere with viral nucleic acid replication. An emerging subset of antiviral nucleosides with promising broad-spectrum antiviral activity are the imino-C-nucleosides (iminovirs).2,3 One such nucleoside – Galidesivir (Immucillin A, BCX4430) – is an adenosine analogue possessing activity against multiple RNA virus families.2,4 The synthesis of iminovirs is a complex process requiring the addition of a metalated heterocycle to an activated iminoribitol acceptor.3,5,6 By preparing a library of synthetic iminovirs, closely related to Galidesivir but with modified nucleobases, we identified an analogue containing the 4-aminopyrrolo[2,1-f][1,2,4-triazine] nucleobase found in Remdesivir exhibited submicromolar inhibition of multiple strains of influenza A and B viruses, as well as members of the Bunyavirales order.3 This promising compound was assessed against influenza A (H1N1) in preliminary in vivo studies in BALB/c mice, where it displayed significant toxicity necessitating further development. Through synthetic modification, attempts to lower the intrinsic toxicity of this molecule have delivered new iminovirs with selective anti-flavivirus activity in the low micromolar range, with improved safety profiles in vitro. Work is ongoing to further develop these promising new antiviral leads.

222. VERAtide: the universal tool to enhance the efficacy of neutralizing antibodies

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Lipid envelope disruption is one of the promising antiviral strategies because it has three major advantages: broad spectrum of therapeutic coverage, no emergence of drug resistance and low cytotoxicity. Physicochemical properties are known to be important for activities of pore-forming antimicrobial peptides. We have searched for peptides with a particular range of physicochemical properties from Swiss-Prot protein DB and discovered novel antiviral peptides, named as VERAtides (virus envelope-rupturing antiviral peptides). Our own VERAtides have liposome rupture activities with diameter-dependent and concentration-dependent manners. In addition, VERAtides showed potent neutralization activity with IC50 values in the nanomolar range against various viruses and the selectivity index is > 10,000. Moreover, by fusing VERAtides to antibodies which recognize viral envelope proteins, we can improve dramatically antiviral activities of neutralizing antibodies. Therefore, antibody-VERAtide fusion could be the universal tool to enhance the efficacy of neutralizing antibodies.

223. A Biocompatible, Virucidal Zwitterionic Antiviral Polymer Reduces Chikungunya Virus Infection in Murine Models

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Chikungunya virus (CHIKV) is a clinically relevant arbovirus that causes a debilitating, febrile disease with new outbreaks emerging globally. Yet, no effective anti-CHIKV therapeutics are available. Disinfectants that irreversibly inhibit viruses via an extracellular virucidal mechanism are effective against CHIKV but are highly cytotoxic, limiting their use to cleaning of solid surfaces. We have developed a novel zwitterionic polymer (Pol1) that has demonstrated biocompatible antiviral properties and an irreversible inhibitory mechanism against CHIKV, with an IC50 of 0.56 μM. Pol1 also inhibits virus infection in the presence of serum proteins, with an IC50 of 1.38 μM, indicating its effectiveness in a blood environment. In murine models, daily intraperitoneal injection of Pol1 at 10 mg/kg for 7 days showed biocompatibility and reduced CHIKV-induced pathologies, including viremia and joint swelling. Further investigations into the immunopathological responses to Pol1 need to be determined, yet its antiviral properties characterise it as a promising anti-CHIKV therapeutic.

224. LHF-535 and favipiravir synergize to protect against experimental Junín virus infection and disease

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Argentine hemorrhagic fever, caused by Junín virus (JUNV), is the most common arenaviral hemorrhagic fever in South America. The disease has a case fatality rate of 15-30% in untreated patients. While early intervention with immune plasma is effective, dwindling stocks and limited availability outside of Argentina underscores the need for new therapeutics. Ideally, these would be broadly active agents effective against all the pathogenic arenaviruses. The fusion inhibitor LHF-535 and the nucleoside analog favipiravir have shown promise in animal models of Lassa fever, a disease endemic in parts of Africa and the most prominent of the arenaviral hemorrhagic fevers. Versus JUNV, a high dose of favipiravir is required to achieve complete protection in the gold-standard guinea pig infection model. Here, we demonstrate strong synergy through the coadministration of LHF-535 with a suboptimal dose of favipiravir, resulting in complete protection from lethal JUNV disease in guinea pigs. As a monotherapy, LHF-535 only delayed the onset of severe disease in the animals. The benefit of the drug combination was also evident by the

absence of infectious virus in tissues and serum compared to guinea pigs treated with vehicle placebos. Thus, combined targeting of virus-host membrane fusion and viral RNA synthesis with pan-arenaviral LHF-535 and favipiravir may expand their indication beyond Lassa fever and provide a significant barrier to drug resistance.

225. In vivo Evaluation of the Antiviral Efficacy of Subcutaneous Nafamostat Formulated with Glycyrrhizic Acid against COVID19

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The ongoing COVID-19 pandemic underscores the essential need for effective antiviral agents and vaccines. Drug repurposing, involving the modification of existing medications, offers a promising strategy for expediting the development of novel therapeutics. In this study, we developed a new drug, MDB-MDB-601a-NM, by modifying the existing drug Nafamostat with the incorporation of glycyrrhizic acid. We assessed the pharmacokinetic profiles of MDB-601a-NM and Nafamostat in Sprague-Dawley rats, revealing rapid clearance of Nafa-mostat and sustained drug concentration of MDB-601a-NM after subcutaneous administration. Single-dose toxicity studies indicated potential adverse effects and persistent inflammation at the injection site with high doses of MDB-601a-NM. Moreover, using a transgenic mouse model, we evaluated the efficacy of MDB-601a-NM against SARS-CoV-2 infection. Mice treated with MDB-601a-NM at 60 mg/kg and 100 mg/kg demonstrated enhanced protection with reduced weight loss and improved survival rates compared to the nafamostat-treated group. Histopathological analysis showed dose-dependent improvements in tissue pathology and increased inhibitory efficacy in MDB-601a-NM-treated groups. Importantly, no viral replication was observed in brain tissue upon administration of 60 mg/kg and 100 mg/kg of MDB-601a-NM. Our synthesized compound, MDB-601a-NM, combines Nafamostat with glycyrrhizic acid, showing promising efficacy against SARS-CoV-2 infection. Its sustained drug concentration after subcutaneous administration and dose-dependent improvements makes it a promising therapeutic option.

226. Development of a multiplex screening assay for identifying novel antiviral targets involved in ebolavirus proteolysis

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Ebolavirus entry into target cells requires host cell proteolysis of the viral glycoprotein (GP). This cleavage facilitates removal of the glycan cap and mucin-like domain leading to exposure of the receptor binding domain (RBD). Endosomal cleavage is primarily mediated by cathepsin B (CTSB), however CTSB knock-out mice are still susceptible to infection and CTSB inhibitors have failed to show potency in murine models of ebola. Other than the closely related cathepsin L, the identify of additional proteases able to activate ebolavirus remain unknown. Using VSVΔG/Ebola GP chimeric virus, we have selected CTSB-independent mutants via both a broad-spectrum CTSB/L inhibitor and CTSB-knockout cells. This has identified mutations in the N-terminus of GP1 and fusion machinery of GP2, similar to previously described escape mutants and naturally occurring variants. However, we also identified novel mutations in the RBD and close to the CTSB cleavage site. All of these CTSB-independent GPs retain sensitivity to broad-spectrum protease inhibitors suggesting proteolysis is still required. Thus, we developed a multiplex lentiviral screening assay to simultaneously interrogate multiple CTSB-independent GPs in order to identify novel protease targets. Luciferase reporter backbones with distinct emission spectra were used to allow deconvolution of signal from a single substrate, allowing savings in cost and time. Screens included libraries of protease inhibitors and genetic knockout libraries. These studies will identify additional proteases required for ebolavirus entry in vivo, allowing the development of targeted protease inhibitor cocktails to efficiently inhibit infection.

227. From Mystery to Mastery: Merkel cell polyomavirus Research and the Promise of siRNA Genomic Silencing

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Merkel cell polyomavirus (MCPyV) is a significant contributor to the development of Merkel cell carcinoma (MCC), an aggressive skin cancer with high recurrence and low survival rate. The exact routes through which MCPyV is transmitted remain unclear, although several factors may trigger its development. Research on MCC is hampered by the scarcity of reliable cell lines, tumour samples, and animal models. The incidence of MCC among Caucasian individuals also presents difficulties in recruiting participants from other ethnic groups for research studies. Current diagnostic methods may be impeded by inexperience, and the pharmaceutical industry's emphasis on diseases affecting larger patient populations may discourage investment in customized treatments for MCC. In the near future, small interfering RNA (siRNA) delivery systems that specifically target MCPyV-infected cells may be employed for MCC treatment. Clinical trials evaluating the safety and efficacy of siRNA therapies targeting MCPyV in patients with MCC are necessary to translate preclinical findings into clinical practice.

This study aims to use lipid-based nanoparticles to deliver siRNAs that target T antigens of MCPyV in vitro and in vivo. The research also aims to answer specific questions, such as the design of siRNAs that specifically target the T antigen of MCPyV, the evaluation of transfection efficiency, the measurement of cell viability, the delivery of siRNA candidates via lipid nanoparticles in vitro, and the delivery of siRNA candidates using developed LNPs in transgenic mouse models with MCPyV-driven MCC.

228. Nucleocapsid- and Glycoprotein 38 targeting monoclonal antibodies protect mice against Crimean-Congo hemorrhagic fever virus

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Antibody-based medical countermeasures are powerful tools to combat viral disease. Some evidence suggests that antibodies can protect humans against lethal disease caused by Crimean-Congo hemorrhagic fever virus (CCHFV), but the protective efficacy of antibodies has only recently begun to be explored in relevant animal models. Here we report on our development of protective monoclonal antibodies (mAbs) targeting the glycoprotein 38 (GP38) and nucleocapsid protein (NP) of CCHFV. When given prior to challenge, protection in mice with the anti-GP38 antibody called mAb-13G8 required complement activity, but not Fc-receptor functionality. When given on day 3 post-infection, mAb-13G8 was capable of therapeutic protection, preventing lethal infection in ~75% of infected mice. Because complement activity was required for protection, the localization of the GP38 molecule was examined and found in addition to being secreted, it also localizes to cellular plasma membranes and the envelope of CCHF virus-like particles. The CCHFV NP is highly conserved between CCHFV strains and functions as a vaccine target. We found that anti-NP monoclonal antibody (mAb-9D5) protected mice against lethal CCHFV infection and resulted in a significant delay in mean time-to-death in mice that succumbed to disease. However, when given post-infection, mAb-9D5 did not exhibit robust protection. Curiously, NP was detected on the surface of infected cells; however, antibody protection was independent of Fc-receptor functionality and complement activity. This work provides critical insight into antibody-based therapeutics against CCHFV and will help guide products targeting this important pathogen.

230V. The Design, Synthesis, and Antiviral Evaluation of a Series of Flex-2'-deoxy-2'-fluoro-2'-methyl Nucleos(t)ide Analogues

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The 2'-deoxy-2'-fluoro-2'-methyl-beta-D-ribofuranose sugar moiety has shown great potential to serve as a scaffold for robust nucleos(t)ide antiviral therapies, from compounds like the FDA approved treatment for HCV, Sofosbuvir, to the Phase III SARS-CoV-2 compound, Bemnifosbuvir. The Seley-Radtke lab has developed and utilized a novel flex-purine base modification to develop a new kind of flexible nucleos(t)ide analogue, called Fleximers, with unique capabilities. Application of the Fleximer base modification has produced analogues that have shown stronger as well as broader antiviral activity compared to the parent analogues that do not feature flexbases. The work herein will describe the application of this flex-purine base modification, consisting of a five- and a six-membered ring conjugated by a single carbon-carbon bond, to the 2'-deoxy-2'-fluoro-2'-methyl-beta-D-ribofuranose sugar scaffold. This work will probe to see how this flex base modification can possibly affect the antiviral properties compared to that of their rigid, fused-base counterparts.

250V. Preclinical Services available through NIAID's Division of Microbiology & Infectious Diseases (DMID)

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The National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), of the Department of Health and Human Services supports research for the understanding of microbiology and immunology leading to the development of vaccines, therapeutics, and medical diagnostics for the diagnosis, prevention, and treatment of infectious and immune-mediated diseases. Within NIAID, the Division of Microbiology and Infectious Diseases (DMID) specifically facilitates research to understand, control and prevent diseases caused by human infectious agents except for HIV. DMID not only manages research grants, cooperative agreements, and contracts to support basic, applied, and translational research, but also provides services to help advance development of therapeutics, vaccines, and diagnostics via an array of preclinical and clinical services to support multiples stages of the product development pipeline. These free-of-charge services, include In Vitro Assessment for Antimicrobial Activity, Preclinical Models of Infectious Diseases using in vivo animal testing (small and large animals) and Complex In Vitro Systems, such as Microphysiological Systems (MPS) screening, B.E.I. Resources Repository, Therapeutic Development Services, and Vaccine Development Services (https://www.niaid.nih.gov/research/resources?f%5B0%5D=division%3A12). Data from these activities may be used by the requestor (open to extramural investigators in academia, industry, and Government) to assist in future funding initiatives, determine key go/no-go decisions and/or facilitate regulatory submissions, patents, and other intellectual property applications for their potential products.

251V. Withdrawn

252V. Establishment of a lethal model of Nipah virus infection through transient immunosuppression of wild-type mice

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Nipah virus (NiV) is a zoonotic pathogen that can cause fatal respiratory and neurological disease in humans, with case fatality rates up to ~70%. Several small animal models of infection have been established, including hamsters, ferrets, and IFNAR-/- mice. An alternative to IFNAR-/- mice, the immunosuppression (IS) model, uses a monoclonal antibody (mAb 5A3) that targets the IFNAR-1 subunit of the IFN-alpha/beta receptor to transiently suppress type I IFN responses in wild-type mice, making otherwise immunocompetent animals susceptible to lethal viral infection. Here, we investigated whether an IS model could be developed for NiV. C57BL/6J mice received 2.5 mg of mAb 5A3 on one of the following schedules: 0, 0/+4, or 0/+4/+7 days post infection (dpi). Both IS mice and IFNAR-/- mice, for comparison, were challenged intraperitoneally with NiV Malaysia (NiV-M; 1.0 x 10^7 TCID50). Mice were euthanized serially at 4 or 6 dpi, when meeting euthanasia criteria, or at study end (28 dpi). NiV-M was 75% and 62.5% lethal in IFNAR-/- and IS mice (both 0 and 0/+4 dpi cohorts), respectively. Lethality decreased in IS mice given mAb 5A3 at 0/+4/+7 dpi (12.5%). Both mouse models resulted in similar respiratory and neurological signs consistent with human disease. Tissues including liver, spleen, kidney, heart, lung, eye, and brain, as well as mucosal swabs, were collected for determination of viral load from all animals. Plasma was also collected and used for immunologic analyses. Here, we establish a new transiently immunosuppressed mouse model of NiV infection for use in medical countermeasure studies and to further investigate host factors associated with disease outcome.

254V. Targeting Flaviviruses Replication by 5-Aminotriazole-amide Inhibitors

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Flavivirus, a genus of Mosquito-borne viruses, includes West Nile Virus (WNV), Dengue Virus (DENV), and Zika virus (ZIKA) which have been particularly problematic diseases in the developing world. The four serotypes of the dengue virus are estimated to cause 50-100 million human infections annually. Currently there are no approved treatments for flaviviruses, indicating a critical need for the development of antiviral agents capable of targeting these viruses. We developed a high throughput screen against DENV-2 and DENV-4 and screened 200K unique compounds from which 11 hits were identified and then reconfirmed in the antiviral and virus titer reduction (VTR) assays. Amongst the 11 hits, SRI-45337 exhibited antiviral inhibition against DENV-2 (EC90 = 1.7 μM, VTR of 3.1 logs) with no cytotoxicity up to 30 μM in HEK293 cells. In addition, SRI-45337 showed acceptable solubility (14.3 μM) and poor metabolic stability which needs further optimization [t1/2 = 12.6 min in human (HLM) and t1/2 = 27.7 min in mouse liver microsomes (MLM)]. A structure-activity relationship (SAR) campaign was initiated on the SRI-45337 series to improve antiviral potency and drug-like properties. These efforts led to the identification of SRI-46848, which showed improved antiviral activity against DENV-2 (EC90 = 0.4 μM, VTR of 2.9 logs) and a three-fold improvement in in vitro microsomal stability (HLM t1/2 = 43 min, MLM t1/2 = 65.8 min). The SAR studies and biological results, including in vivo data of SRI-46848 in a murine model against DENV, will be discussed.

255V. Potent antiviral activity of Plitidepsin against Ebolavirus

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Ebolavirus infection results in a severe and rapidly progressive disease (Ebolavirus disease, EVD) with a high mortality ranging from 40-80%. Treatment of infected patients is mainly supportive. Medical countermeasures for EVD are limited and consist in a preventive vaccine and two monoclonal antibodies that have demonstrated partial clinical efficacy. Plitidepsin is a marine cyclic peptide developed as antitumoral that targets the host protein eEF1A (eukaryotic translation elongation factor 1 alpha) and has recently shown high antiviral activity against SARS-CoV-2. We have tested the antiviral activity of Plitidepsin on Ebolavirus (EBOV) using a minigenome system that allows the study of viral genome replication and transcription without BSL-4 requirements. Remdesivir, an inhibitor of viral RNA polymerases was used as a control. Two independent experiments were performed by triplicates and 50% inhibitory concentration (IC50) and 95% confidence intervals were obtained by Graphpad Prism v8. Plitidepsin was highly active against Ebolavirus infection (IC50 for EBOV 6.27 nM, 95% CI: 5.80-6.78 nM), exhibiting a >5X potency as compared with Remdesivir (IC50 36.22 nM, 95% CI 34.23-38.33). These results of antiviral activity of Plitidepsin along with the safety and toxicity profile already stablished in human studies warrant the investigation of Plitidepsin as a potential clinical treatment alternative. Due to the mechanism of action of Plitidepsin is highly likely that antiviral activity is also conserved for other member of the Filoviral family for whom no vaccine or treatment is available. Those studies are currently in progress

256V. Diaryl Ethers in Double Combinations with Pocapavir against Poliovirus 1 and Coxsackievirus B4

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Enteroviruses (EV) are widespread and economically significant, making them a significant challenge for researchers. The current absence of effective clinical available antiviral drugs for the treatment of EVs highlight the urgency and significance of developing antiviral agents. The resistance occurring after monotherapy with a certain anti-enteroviral drugs makes it reasonable to focus interest on combined administration of antivirals. We investigated the effects in cell culture based on combination of capsid-binding inhibitor pocapavir and some newly synthesized diethyl ethers. Double combinations by newly synthesized diethyl ethers, derivatives of 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (MDL-860) – 2-(2,6-difluorophenoxy)-5-nitrobenzonitrile (CB-109) and 2-(2,4,6-trifluorophenoxy)-5-nitrobenzonitrile (VGA-12) with pocapavir were tested on HEp-2 cells for their activity against Poliovirus 1 (PV-1) and Coxsackievirus B4 (CV-B4). Antiviral combination effects due to drug–drug interaction were examined by relying on the three-dimensional model developed by Prichard and Shipman (1990) by using

the program MacSynergyTM II. The combinations of CB-109 or VGA-12 with pocapavir were additive, while the combination of MDL-860 and pocapavir demonstrated moderate synergistic effect. Results are similar for PV-1 and CV-B4 (55.9 and 56.1 synergy, respectively). All combination studies included evaluations of cell viability, and none of the tested combinations showed cytotoxicity within the range of drug concentrations examined.

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300. SARS-CoV-2 PLpro Inhibitors for COVID-19 Therapy

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The pandemic caused by the novel SARS-CoV-2 coronavirus led to a large public health burden. There are a limited number of approved vaccines and antivirals to prevent and treat COVID-19 infections. Despite this, the constant emergence of SARS-CoV-2 variants has reduced the efficacy of available vaccines and drug treatments. In addition to vaccines, antiviral therapeutics have been pivotal in reducing SARS-CoV-2 infections and COVID-19 associated deaths. The papain-like protease (PLpro), an essential viral protease that is highly conserved among coronaviruses, presents an attractive target for antiviral drug development. Currently, few potent and validated PLpro inhibitors exist. This scarcity is due to the featureless binding pockets at the "gly-gly" recognizing P1 and P2 substrate-binding sites, posing a significant challenge in developing potent inhibitors. In our previous research, we identified a series of potent PLpro inhibitors, some of which bind to a newly discovered region on the PLpro, the "BL2 groove". Building on this research, we developed a new series of covalent inhibitors. These inhibitors feature a linker that mimics the gly-gly sequence, enabling them to penetrate the gly-gly channel that hosts the catalytic cysteine and form a covalent bond. Several compounds in this series have demonstrated an enzymatic potency below 10 nM. A few of these molecules have shown similar or superior efficacy against the infectious SARS-CoV-2 strain WA1/2020 compared to nirmatrelvir, with an EC90 < 0.6 μM. Preliminary pharmacokinetic studies have validated the feasibility of oral administration. Ongoing animal studies are aimed at determining their in vivo efficacy.

301. Nsp8-TP25 Peptide as a Promising Therapeutic Agent Targeting the SARS-CoV-2 RdRp Complex

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) replication involves RNA-dependent RNA polymerase (RdRp) Nsp12 for viral genome replication but requires Nsp7 and Nsp8 for efficient transcription and replication. Recent studies show that Nsp8 stabilizes the RdRp machinery, enhancing its processivity. The increased interaction of Nsp12-Nsp8 complex enhances viral replication and transmission among SARS-CoV-2 VOCs. Based on these studies, we designed a set of peptides from the sequence of SARS-CoV-2 Nsp8 interaction interface with Nsp12 fingers domain and demonstrated the antiviral activity in vitro and in vivo. Briefly, we evaluated Nsp8-TP25's ability to inhibit RdRp function by interfering with vRNP complex by performing a cell-based RdRp activity reporter gene assay of SARS-CoV-2. Results showed a dose-dependent inhibition of polymerase activity. Furthermore, Nsp8 peptide inhibited SARS-CoV-2 replication in Vero E6 cells, confirming the minigenome assay data. We then sought to demonstrate the therapeutic or prophylactic potential of Nsp8-TP25 against SARS-CoV-2 in vivo. Balb/c mice were intranasally administered with Nsp8-TP25 (25mg/kg BW) which showed strong antiviral activity, protection against weight loss, and death after maSARS-CoV-2 Wuhan strain challenge. Moreover, prophylactic or therapeutic administration of Nsp8-TP25 peptide reduces pathogenicity and infectious viral titers in nasal turbinates and lungs. Thus, exogenous Nsp8-based peptides may interact with Nsp12 forming Nsp12-Nsp8 binding-defective complex, depleting the pool of Nsp12 proteins necessary for active RdRp complex formation, leading to the allosteric inhibition against SARS-CoV-2.

302. "A potent small molecule inhibitor of Receptor Binding Domain and ACE2 receptor interaction"

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SARS-CoV-2 is a novel beta-coronavirus that follows the previously identified SARS-CoV and MERS-CoV, which causes viral respiratory disease that involves the airway and respiratory tract, leading to severe respiratory distress, immunological activation, and related morbidity and mortality. SARS Cov-2 invades host cells through interaction, recognition, and binding of Spike protein with the peptidase domain of the human receptor ACE2 (angiotensin-converting enzyme 2). ACE2 is a cell surface membrane protein expressed in human alveolar epithelial cells that enables COVID-19 infection. ACE2 domains contain a signal peptide, a transmembrane domain, and an intracellular domain. During infection, the extracellular peptidase domain of the ACE2 receptor binds to the Receptor Binding Domain of the spike protein, a surface protein on SARS-CoV-2. Docking studies of pyridine Dicoumarol molecules with the RBD region of spike protein have been done using the AutoDock tool. Based on docking results, the molecules UHAKKM-10, UHAKKM-12, and UHLMT-79 showed binding energy of -7.09, -7.65, and -7.98 respectively, suggesting significant binding affinity. The molecules having significant binding affinity have been analyzed in in-vitro studies for RBD and ACE2 interaction interference using ELISA and immunofluorescence. These studies confirm that molecules UHAKKM-10, UHAKKM-12, and UHLMT-79 possess significant activity in inhibiting RBD and ACE2 receptor interaction with IC50 values of 16.92 µM, 6.058 µM, and 1.072 µM, respectively. In summary, pyridine derivatives may form new pharmacophores for the development of inhibitors for Cov-2 entry to ACE2 cells.

303. Gingival vaccination as an antiviral vaccination strategy: Prospects for elderly vaccination

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Ageing causes the gingival crevice (GC) to widen, thereby, allowing periodontopathic bacteria to enter the gingival mucosa and, possibly, have a systemic effect. Interestingly, Langehans cells found in the sulcular epithelium (along the GC) remain functional and, surprisingly, actually increases with age ascribable to the age-related build-up of dental plaque. This would suggest that as the GC becomes wider with age, target antigens can easily penetrate and induce an immune response. However, the potential of a using the GC as a possible vaccination route has never been explored. Throughout this study, we used young (20 week-old) and elderly (77 week-old) rats for comparison of immune response. We simulated xanthan gel-encapsulation of representative antigens (Dengue virus envelope subtype-2, Influenza A nucleoprotein, and Influenza A H5N1 hemagglutinin) in order to verify that target epitopes were not blocked. Subsequently, we compared the antibody titer among gingival-vaccinated rats (old and young) and, likewise, we evaluated the antibody titer produced via the gingival route as compared to other vaccination routes (intradermal, oral, sublingual). Lastly, we orally supplemented two sets of rats with two different antigen doses: mixed low-dose (50 ug mL-1 per antigen) and separated high-dose (100 ug mL-1 per antigen). Rat blood serum was collected for further downstream analyses. Our results showed the following: (1) higher amounts enter old rats via oral-supplementation; (2) 100 ug/mL is the optimal concentration for xanthan gel-encapsulation; and (3) gingival-vaccinated old rats have higher antibody titer as compared to young rats and other vaccination routes.

304. Potent Anti-COVID-19 Agent, GMS007, Made of Stable and Cell-Permeable Peptide Nucleic Acid (PNA)

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Despite the development of a safe and effective COVID-19 vaccine, COVID-19 continues to mutate and spread. Currently, PAXROVID and Remdesivir are recommended as treatments, but these treatments have concerns about drug-drug interactions and doubts about their effectiveness against variants. So the development of novel therapeutic options are still needed. Antisense oligomers (ASO) have excellent potential for treating diseases, especially for antivirals, because they can inhibit gene expression such as transcription and translation in a sequence-specific manner. However, their applications for therapy are pretty much limited due to their lack of in vivo stability and cell permeability. Here, we introduce the potent anti-COVID-19 ASO made of

very stable and excellent cell-permeable peptide nucleic acid (PNA). These molecules targeted highly conserved genome sequences of COVID-19, and their binding affinity towards COVID-19 complementary genome is less than pM range. In an in vitro assay system using Caco2 or Vero6 cells, our series of ASOs targeted complements to the COVID-19 genome showed better activity in reducing viral RNA levels than PAXLOVID. The EC50 value of representative compound GMS-007 was 200 nM in Vero6 cells, whereas the EC50 value of PAXLOVID was 550 nM. During the in vitro assay, any of the delivery tools were not utilized. (or No delivery tools were used during the in vitro assay.) This presentation describes the basic chemical structure of our technology and its possible mode of action (MOA) for cell permeability, the design of GMS-007 for anti-COVID-19, and the in vitro activity of GMS-007 compared to PAXLOVID.

305. Evaluation of Major Components of Green Plants, as Potential Therapeutics for SARS-CoV-2 variants

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Although several vaccines and antiviral drugs against SARS-CoV-2 are currently available, control and prevention of COVID-19 through these interventions is limited due to inaccessibility and economic issues in some regions and countries. Moreover, incomplete viral clearance by ineffective therapeutics may lead to rapid genetic evolution, resulting in the emergence of new SARS-CoV-2 variants that may escape the host immune system as well as currently available COVID-19 vaccines. Here, we report that phytochemicals extracted from Chlorella spp. and Psidium guajava possess broad-spectrum antiviral activity against a range of SARS-CoV-2 variants. Through chromatography-based screening, we identified four bioactive compounds and subsequently demonstrated their potential antiviral activities in vivo. Interestingly, in animal models, treatment with these compounds significantly attenuates SARS-CoV-2-induced proinflammatory responses, demonstrating their potential anti-inflammatory activity. Taken together, we identified plant-derived bioactive compounds possessing antiviral properties against a broad range of SARS-CoV-2 strains and variants. Thus, our data provide evidence that phytochemicals from edible plants can be an ideal candidate in tackling the broad spectrum of SARS-CoV-2 strains and variants for both therapeutic and prophylactics purposes.

306. In Silico Identification and In Vitro Validation of Repurposed Compounds Targeting the RSV Polymerase

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Respiratory Syncytial Virus (RSV) is the top cause of infant hospitalization globally, with no effective treatments available. Researchers have sought small molecules to target the RNA-dependent RNA Polymerase (RdRP) of RSV, which is essential for replication and transcription. Based on the cryo-EM structure of the RSV polymerase, in silico computational analysis, including molecular docking and the protein-ligand simulation of a database that is currently undergoing phases 1-4 of clinical trials and has resulted in the top ten repurposed compound candidates against the RSV polymerase, including Micafungin, Totrombopag, and Verubecestat. We performed the same procedure to evaluate 18 small molecules from previous studies and chose the top four compounds for comparison. Among the top identified repurposed compounds, Micafungin, an antifungal medication, showed significant inhibition and binding affinity improvements over current inhibitors such as ALS-8112 and Ribavirin. We also validated Micafungin's inhibition of the RSV RdRP using an in vitro transcription assay. These findings contribute to RSV drug development and hold promise for broad-spectrum antivirals targeting the non-segmented negative-sense (NNS) RNA viral polymerases, including those of rabies (RABV) and Ebola (EBOV).

307. Targeting viral RNA as new approach for antivirals: from in silico to cells

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We are lacking antivirals for different viruses. In addition to targeting viral protein, focusing on viral RNA is a promising strategy to address this gap in antiviral therapeutics. We previously showed geneticin's inhibition of various SARS-CoV-2 variants by targeting its -1 programmed ribosomal frameshift. This mechanism involves a pseudoknot, an RNA structure common in coronaviruses but uncommon in humans. To identify more potent inhibitors, we conducted virtual screening with a drug-like library on an RNA ensemble derived from molecular dynamics, using a refined SARS-CoV-2 pseudoknot structure. One of the hits demonstrated reduction of SARS-CoV-2 frameshift and exhibited antiviral activity in the micromolar range. However, cellular studies revealed a different SARS-CoV-2 RNA folding, driving our focus on identifying molecules binding to alternative structures that could result in a conformational block, preventing the formation of the pseudoknot. Consequently, we are selecting potential inhibitors by virtual screening and using a refined dual luciferase construct in which the RNA can fold as identified in the cells. This novel drug development strategy is applicable to various viruses. We are currently employing this approach on rhinoviruses by determining the predominant RNA cellular structure with DMS probing, selecting druggable pockets with antisense oligonucleotides, and using in silico screening to identify potential drug candidates. Overall, targeting viral RNA is an unexplored field with promising therapeutic opportunities.

308. Discovery of Nanosota-2, -3, and -4 as super potent and broad-spectrum therapeutic nanobody candidates against COVID-19

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Nanobodies are single-domain antibodies derived from camelid animals. Here, we discovered three anti SARS-CoV-2 nanobodies, namely, Nanosota-2, -3, and -4, from an alpaca immunized with SARS-CoV spike protein. We further characterized the antiviral activities of these Fc-tag-fused nanobodies. Notably, Nanosota-2 inhibits the prototypic SARS-CoV-2 strain in vitro (with an IC 50 of 2 pM) and in mice (at a dosage of 4 mg/kg or administered 18 hours post-challenge). These potency metrics are the best among known SARS-CoV-2 entry inhibitors. Moreover, Nanosota-3 effectively inhibits the omicron variant, both in vitro and in mice, regardless of the administration route (intraperitoneal or intranasal). Furthermore, Nanosota-3 has been biochemically engineered to inhibit both early and currently circulating subvariants of omicron. Additionally, Nanosota-4 uniquely inhibits both SARS-CoV-1 and SARS-CoV-2. Cryo-EM data revealed that the three nanobodies bind to functionally critical and non-overlapping regions in the spike protein. Given their cost-effectiveness, ease of adaptation to new viral strains, and potential use as inhalers, the Nanosota series are powerful therapeutic tools against coronavirus pandemics.

309. A New Cell Culture-based Assay IRINA to Assess Antiviral Susceptibility of Seasonal and Variant Influenza Viruses

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Antiviral phenotypic testing is part of influenza virologic surveillance, which supplements sequence-based analysis of drug resistance markers. Phenotypic assays are essential when such markers are unknown or when novel viruses emerge. Baloxavir is the newest FDA-approved influenza antiviral that targets the endonuclease activity of the polymerase acidic (PA) protein. Here, a streamlined cell culture-based assay IRINA (Influenza Replication Inhibition Neuraminidase-based Assay), recently developed by us, was applied to monitor baloxavir susceptibility. PA sequences of seasonal and swine-origin (variant) viruses were obtained by next generation sequencing and analyzed for known and suspected markers of decreased baloxavir susceptibility. IRINA was used to determine baloxavir EC50 for seasonal viruses collected in 2022-23, and 10 variant viruses of A(H1N1)v, A(H1N2)v, and A(H3N2)v subtypes collected in the United States in 2020-23. None of the viruses had known PA markers, except for one seasonal A(H3N2) virus with PA-I38T. In IRINA, they displayed normal inhibition by baloxavir as their EC50s were within 3-fold of the (sub)type-specific median. The median EC50s of A(H1N1)pdm09 (n=276) and A(H3N2) (n=184) were similar, 0.7nM. B/Victoria viruses (n=143) displayed a higher median EC50, 4.5nM, as seen in other assays. The PA-I38T in the A(H3N2) virus conferred 80-fold decreased baloxavir susceptibility. The EC50s of the variant viruses ranged from 0.2 to 1.4nM, which are similar to those of seasonal influenza A viruses. IRINA was successfully applied to monitor baloxavir susceptibility of influenza viruses and is being implemented by other surveillance laboratories.

310. Intriguing Structure of the Influenza A Virus Genome – Are G-quadruplexes Potential Targets for Antivirals?

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Influenza A virus (IAV) causing pandemic outbreaks became an important research subject. Despite the high variability of its genome, viral RNA (vRNA) structure possesses features that remain constant between strains. The significance of the vRNA secondary structure in the viral life cycle has been already demonstrated. Among the vRNA structures are G-quadruplexes (G4s) formed within G-rich sequences, i.e. PQSs. G4s have various functions during the replication cycle and thus are investigated as potential therapeutic targets. We searched the IAV genome for the presence of PQSs, studied their ability to fold into G4s, and their potential role in the viral life cycle. Using bioinformatics tools, we identified twelve PQS motifs within the IAV vRNA. Then, by biophysical methods, we determined their propensity to form G4s. To this end, we used spectroscopic techniques and reverse transcription stop assay. Additionally, we performed biological studies in the cell culture of the influence of G4-specific ligands, TMPyP4 and TMPyP2, on the replication within the IAV minireplicon system. Our results revealed that three PQSs form stable RNA G4s within segments encoding viral polymerase complex proteins and G4-specific ligands can stabilize G4s restricting product synthesis. The biological studies showed that TMPyP4 effectively inhibits IAV replication and is a more potent inhibitor than TMPyP2. We concluded that G4s are present within the IAV genome and can be targeted by specific ligands. What is more, via G4 stabilization, viral replication can be effectively inhibited. All our findings suggest that selected IAV PQS motifs can serve as potential novel antiviral therapeutic targets.

312. A natural compound and its synthetic derivative: towards new potent pan-coronavirus antiviral agents

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The COVID-19 pandemic, caused by SARS-CoV-2, has highlighted the lack of specific antivirals available against human coronaviruses (HCoVs). To get rid of SARS-CoV-2 and face future emerging HCoVs, efficient antiviral treatments are necessary. Plants are a valuable source of compounds with higher structural diversity than chemically synthetic ones. Our global strategy is to identify pan-coronavirus antivirals. Antiviral screening of natural compounds against the low pathogenic HCoV-229E, revealed molecules of the class of phloroglucinol derivatives (PDs) as potent inhibitors. Antiviral activity of the lead natural compound of the family (PD-1) was also shown against the severe HCoVs, SARS-CoV and MERS-CoV, and several SARS-CoV-2 variants, with no toxicity at active concentrations (IC50 [1.5-6.0] µM, and CC50 [80-100] µM, in vitro). The structural diversity of PDs was increased by producing a series of synthetic analogous compounds using a structure-activity relationship approach. A new undescribed synthetic compound, AH-62, showing similar to reduced IC50 compared to PD-1 was identified. To gain insights in the mechanism of action (MoA), time-of-addition assays were conducted, showing that PD-1 was most likely a replication inhibitor. Finally, the antiviral activity of PD-1 on SARS-CoV-2 and HCoV-229E was confirmed in human primary respiratory epithelial cells grown in air-liquid interface. In conclusion, our interdisciplinary approach reveals two potent pan-coronavirus antivirals, (natural PD-1 and synthetic AH-62). Experiments are under progress to unravel their MoA and to determine their antiviral potency in mouse model.

313. A Favipiravir analogue and chain terminator, active against SARS-CoV-2

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Favivpiravir is an antiviral prodrug of a nucleotide analogue that is ribophosphorylated in the infected cell. Originally developed against influenza virus, it has been shown to be active against several RNA viruses, including SARS CoV-2. Its triphosphate form is incorporated by the viral RNA-dependent RNA polymerase (RdRp), slowing replication and increasing viral mutation-rates, resulting in error catastrophe. While first approved in Japan in 2014 for influenza, its use is highly restricted due to reports of embryotoxicity and teratogenetic effects in animals. As such, it still does not have FDA approval. Furthermore, its viral mutagenic mechanism of action may additionally facilitate the generation of viral variants. Here we explore the incorporation, excision and in vitro anti-SARS CoV-2 activity of a Favipiravir-analogue triphosphate without the fluoro at the base, and carrying a methyl group at the 2' ribose position. We show that this compound is well incorporated by SARS CoV-2 RdRp in the place of GTP, where its ribose modification causes immediate chain-termination and therefore can no longer lead to mutated genomes. Despite the ability of the viral exonuclease to excise the incorporated compound, initial tests with different prodrug forms (ProTides and TriPPPro) indicate that the new compound has activity against SARS-CoV-2 in infected HUH 7.5 cells. .

315. Imiquimod Inhibits the Multiplication of Coronaviruses Through the MAPK/ERK Pathway

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The development of broad-spectrum antivirals against coronaviruses is essential as a complement to vaccination against SARS-CoV-2, and to prepare for future pandemics. Toll-like receptor (TLR) ligands, known for inducing IFN and cytokine production, have been successfully evaluated in various viral infection models. In this sense, we demonstrated direct antiviral activity in vitro and in vivo against RSV of the TLR7 agonist imiquimod (IMQ), independently of the TLR7 pathway and activating the PKA signaling pathway instead. This study aimed to assess the antiviral activity of TLR ligands against SARS-CoV-2 and canine coronavirus (CCoV) in vitro. IMQ postinfection treatment significantly inhibited SARS-CoV-2 and CCoV infections in calu-3 and CRFK cells, respectively. This effect was not observed with other TLR agonists (Pam2CSK4, poly(I:C), LPS, resiquimod, CpG ODN). Moreover, calu-3 cells infected with SARS-CoV-2 and treated with IMQ exhibited reduced levels of viral proteins expression, as determined by Immunofluorescence analysis. Regarding the antiviral mechanism of action of IMQ against coronaviruses, co-treatments with inhibitors or stimulators of the cAMP pathways (PKA and EPAC) and the NF- B pathway had no impact on the antiviral efficacy of IMQ. However, the MEK inhibitor (UO126) attenuated IMQ antiviral activity, and IMQ induced ERK phosphorylation, AP-1 transcriptional activation, and cytokine production. Thus, the antiviral effect of IMQ could be attributed to the MAPK/ERK pathway. In summary, our results demonstrated that IMQ exhibits broad-spectrum antiviral activity against coronaviruses and respiratory viruses, including SARS-CoV-2, via the MAPK/ERK.

316. Disruption of Spike Priming in Virus Entry: Tetrandrine's Potential Against Pan-coronaviruses

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Infections caused by Coronaviruses (CoV) manifest with a range of symptoms and health impacts, spanning from mild to severe. In the pursuit of effective coronavirus treatments, we have identified the traditional Chinese medicine tetrandrine as a pan-coronavirus inhibitor, demonstrating efficacy against HCoV-229E, HCoV-OC43, SARS-CoV-2, and its variants. Tetrandrine specifically hindered the entry process of CoV infections by directly binding to the Spike protein and promoting its degradation. In the presence of drug resistance induction, we identified a mutation in the HCoV-229E S2 region near the TMPRSS2 binding site, and tetrandrine inhibited TMPRSS2 during infection as well. Consequently, tetrandrine's involvement during virus entry interfered with Spike priming by TMPRSS2, leading to the failure of membrane fusion. This study highlights tetrandrine as a natural compound with potent anti-CoV activity, suggesting its potential as a novel therapeutic for Coronaviruses. The underlying antiviral mechanism of tetrandrine firstly opens the possibility of improving its anti-CoV potency and specificity by adjusting its operation between viral Spike protein and TMPRSS2 through medicinal chemistry.

317. Lycorine Inhibits Influenza Virus Replication Through Autophagy Pathways

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Influenza virus can cause seasonal epidemics and occasional pandemic infectious respiratory disease. The great effect of influenza A virus (IAV) on public health highlights the need to develop effective therapeutic antivirals. Lycorine, a Lycoris radiata-derived alkaloid, has been shown to inhibit several virus species based on different mechanisms. However, the antiviral effect and mechanism of lycorine against seasonal IAV still need to be investigated. Lycorine inhibited the replication of IAV in RNA, protein level, and virus titer. Its antiviral activity was related to the downregulation of ATGs, Beclin-1, and LC3B expression. Lycorine also inhibited autophagy through the AKT-mTOR signaling pathway. Consistently, lycorine and 3-MA or Bafilomycin A1 could synergistically inhibit IAV replication by hindering autophagy. Further mechanistic experiments revealed that lycorine had no effect on the formation of autolysosomes in late autophagy. These results revealed that lycorine exhibited antiviral activity against seasonal IAV and the antiviral mechanism by targeting autophagy, thus providing a new supplement for elucidation of the antiviral mechanism of lycorine.

318. Human guanylate-binding protein (GBP)1 inhibits replication of severe acute respiratory syndrome coronavirus type-2 through a mechanism distinct to GBP2 and GBP5

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Severe respiratory syndrome coronavirus type-2 (SARS-CoV-2) infections are associated with significant morbidity and mortality worldwide. Identification and characterisation of intracellular proteins with antiviral activity (known as restriction factors) is a key first step towards the future development of novel host-directed antiviral therapies. In this study, we investigated the antiviral activity of 14 different restriction factors against SARS-CoV-2. Overexpression of human guanylate binding protein (GBP)1 resulted in potent inhibition of the ancestral SARS-CoV-2 strain, as well as against Alpha, Beta, Delta, Omicron BA.1 and Omicron BA.2 variants of concerns (VOCs). Moreover, knockdown or knockout of endogenous human GBP1 resulted in enhanced titres of SARS-CoV-2. GBP1-mediated inhibition of SARS-CoV-2 was dependent on its GTPase activity and occurred after viral genome replication and transcription, but prior to synthesis of nascent viral proteins. While previous studies have reported that human GBP2 and GBP5 restrict SARS-CoV-2 by inhibiting processing of the viral spike protein, pseudoviruses (PVs) expressing the viral spike that were generated in the presence of GBP1 did not show reduced infectivity. Surprisingly, while multiple human GBPs have been reported to inhibit SARS-CoV-2 in vitro, we demonstrate that the presence or absence of the chromosome 3 cluster of mouse GBPs (GBP1/2/3/5/7) did not alter SARS-CoV-2 replication in the upper or lower airways of infected mice. Together, our studies describe the ability of human GBP1 to inhibit SARS-CoV-2 replication by a mechanism distinct to that previously described for GBP5.

319. By interacting with cell junction and polarity proteins, the PDZ Binding Motif of SARS-CoV-2 Envelope protein constitute a major determinant of pathogenicity and appears as an innovative therapeutic target

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The COVID-19 pandemic caused by SARS-CoV-2, has prompted researchers around the world to focus on the pathogenicity of this virus. A better understanding of the molecular mechanisms involved in the virulence of SARS-CoV-2 is necessary for the development of therapies, to mitigate or prevent the impact of coronavirus infection. The envelope protein E is a structural protein of SARS-CoV-2 which has at the C-terminal end a PDZ Binding Motif (PBM) able to interact with PDZ domains of host proteins. A high-throughput screening of all human PDZ domains revealed a specificity profile of partners of the viral E protein: most of them are involved in the dynamics of cellular junctions (ZO-1/TJP1, MPP5/PALS1, LNX2, PARD3, MLLT4). Targeting cellular junctions and polarity components is a common strategy used by viruses to hijack cell machinery to their advantage and the E protein PBM plays a key role in epithelial barrier disruption during infection. Here we report, using recombinant viruses and in vivo studies how the E PBM plays a major role in SARS-CoV-2 pathogenicity and how PBM-PDZ interactions can constitute a novel therapeutic target. The project is focusing on ZO-1, a PDZ partner for which the PBM present the highest affinity. This

interaction was characterized by structural and functional approaches (X-ray crystallography, thermophoresis, pulldown, immunofluorescence). A ChemioBank of 1000 small molecules was screened using Homogenous Time-Resolved Fluorescence technic (HTRF), identifying 36 hits. Dose-response test, cytotoxicity measurement and in cellulo viral inhibition experiment will allow the selection of a short list of compounds to identify promising molecules.

320. Identification of MARVAS110, a potent antiviral compound for Enterovirus D68 infection

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Enteroviruses are a genus of small RNA viruses that cause diseases in both animals and humans, one of which being Enterovirus D68 (EV-D68). Unlike many other human enteroviruses, EV-D68 infection results in respiratory disease, which could develop into a serious condition termed acute flaccid myelitis, a complication leading to weakness in the muscles and reflexes. To date, there are no approved antivirals for the treatment of EV-D68 infection, highlighting the need to expand the search for antivirals. A phenotypic-based assay was developed to screen for compounds inhibiting EV-D68 infection. Several hits were identified and selected for downstream validation, leading to the discovery of MARVAS110, a kinase inhibitor with potent antiviral activity against EV-D68 infection (IC50: 1.804 μM). Time-of-addition, time-of-removal, and entry bypass assays indicate that the drug likely acts in the post-entry stage of replication, from 4-6 hours post-infection. Western blot and qRT-PCR assays reveal significant decreases in viral protein and viral RNA levels. To further elucidate the mechanism of action of MARVAS110, serial passaging of EV-D68 in the presence of subpotent concentrations of MARVAS110 was carried out. After 18 passages, no resistant mutants were identified, suggesting that the target of MARVAS110 is a host factor. siRNA knockdown studies were conducted on currently known targets of MARVAS110, and results show a possible link to the autophagy pathway. Further work will be performed on MARVAS110 to elucidate its mechanism in EV-D68 infection, with the hope that it would be used as a therapeutic for this disease that remains a recurring global health issue.

321. Mechanism of Action of a 4'-Cyano Modified Nucleotide Analog Against a Platform of Diverse Polymerases of Respiratory RNA Viruses

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Several families of positive- and negative-sense RNA viruses responsible for acute respiratory infections are a significant cause of morbidity and mortality worldwide. The availability of safe and effective broad-spectrum antivirals that could address outbreaks or future pandemics is limited. Here, we studied the mechanism of action of a newly discovered 4'-cyano modified C-adenosine nucleotide analog against a platform of diverse RNA-dependent RNA polymerases (RdRps). The parent nucleoside is herein referred to as GS-646089 and its triphosphate form is referred to as GS-646939. Enzyme kinetics show that the RdRp of human rhinovirus type 16 (HRV-16) and enterovirus 71 (EV-71) incorporate GS-646939 with unprecedented selectivity. GS-646939 is incorporated 20- to 50-fold more efficiently than its natural counterpart ATP. The RdRp complex of respiratory syncytial virus (RSV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) use GS-646939 and ATP with similar efficiency. Influenza B RdRp shows a clear preference for ATP and human mitochondrial RNA polymerase (h-mtRNAP) does not show any significant incorporation of GS-646939. Once incorporated, GS-646939 acts as a chain-terminator, although higher NTP concentrations can partially overcome inhibition for some polymerases. Comparative studies with the active

triphosphate form of the 1'-cyano modified antiviral drug remdesivir reveal different mechanisms of inhibition and differences in the spectrum of inhibition of viral polymerases. In conclusion, the 1'-cyano and 4'-cyano scaffolds of nucleotide analogs provide complementary strategies to target diverse RdRp enzymes of respiratory RNA viruses.

322. Directed Evolution of SARS-CoV-2 Spike Protein Reveals Determinants of Fusion Route and Syncytia Formation

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The spike protein (S) of SARS-CoV-2 dictates the virus entry route preference through plasma membrane or endosome fusion and its ability to form syncytia, playing a key role in viral tropism and pathogenesis. Deep mutational scanning approaches have been valuable in determining the functional effects of mutations in S, however, such approaches are limited to focusing on specific subdomains or on a small subset of residues across S. Here, we used a directed evolution approach of full-length S in the context of replicative recombinant vesicular stomatitis virus pseudotypes to explore the combinatorial mutations that evolve across the full-length protein and impact its functional properties. We selected pseudotypes that evolved under selective pressure on the plasma membrane entry route and determined the effect of mutations that accumulated across S on viral fitness, entry route and kinetics, protease sensitivity and syncytia formation. These findings extend our understanding of the functional interplay within and between the structural domains of S and provide a valuable resource for evaluating the impact of mutations found in circulating variants and predicting possible future variants of concern.

323. Novel SARS-CoV-2 entry inhibitors, 2-anilinoquinazolin-4(3H)-one derivatives, show potency as SARS-CoV-2 antivirals in a human ACE2 transgenic mouse model

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The global impact of COVID-19 has led to extensive fatalities, economic downturns, and the breakdown of public health systems. Despite significant advancements through the development of vaccines and antivirals, the COVID-19 persists with recurring surges. Consequently, there remains an ongoing necessity to create therapeutic agents. In our previous studies, we designed and synthesized an array of novel derivatives of 2-anilinoquinazolin-4(3H)-one, illustrating their inhibitory effects

against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and Middle East respiratory syndrome coronavirus (MERS-CoV) in vitro. Following this, we conducted in vivo experiments using modified compounds specifically formulated for oral administration. These compounds exhibited no toxicity in rats and demonstrated inhibition in virus entry. In our investigation, we explored the in vivo effectiveness of these drug candidates against SARS-CoV-2. Three potential drugs, namely 7-chloro-2-((3,5-dichlorophenyl)amino)quinazolin-4(3H)-one (1), N-(7-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)-N-(3,5-dichlorophenyl) acetamide (2), and N-(7-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)-N-(3,5-difluorophenyl)acetamide (3), were administered orally to hACE2 transgenic mice at a dose of 100 mg/kg. All three drugs increased survival rates and reduced the viral load in the lungs. These findings indicate that these derivatives exhibit in vivo antiviral efficacy comparable to molnupiravir, an approved COVID-19 drug. In conclusion, our data suggest that derivatives of 2-anilinoquinazolin-4(3H)-one show promise as potential oral antiviral drug candidates against SARS-CoV-2 infection.

324. Evaluating SARS-CoV-2 Antiviral Activity Using the Drosophila Midgut Model

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Recent findings have brought to light the Gastro Intestinal (GI) symptoms in COVID-19 patients. Drosophila melanogaster serves as a crucial model for exploring SARS-CoV-2 pathophysiology due to the genetic overlap with humans, where around 90% of human proteins interacting with SARS-CoV-2 are conserved in the fruit fly. Here, we use the Drosophila midgut model to probe the GI pathogenesis caused by SARS-CoV-2. The study reveals the fly midgut's susceptibility to SARS-CoV-2, leading to viral replication, disrupting the gut's epithelial structure, reducing organ size, and altering visceral muscle activity. There was a significant increase in intestinal stem cell proliferation and a reduction in the viability and renewal of differentiated cells, suggesting impaired regeneration. Transcriptome analysis highlighted significant gene expression changes, particularly in lipid metabolism, with SARS-CoV-2 causing lipid droplets accumulation in the midgut's posterior and depletion in the anterior segments. Plitidepsin, a potential COVID-19 treatment, was effective in mitigating these pathogenic symptoms and normalizing lipid droplet distribution. Toxicity and antiviral assays revealed that Plitidepsin's effectiveness and safety are dose-dependent, improving epithelial structure at 10 nM and completely reversing damage at 100 nM. However, Plitidepsin did not address the abnormal contractions of visceral muscles. Moreover, at 10 and 100 nM, Plitidepsin altered visceral muscle structure without infection. This study underscores the Drosophila midgut's values in exploring SARS-CoV-2 GI pathology and evaluating potential COVID-19 treatments and their relevance to future pandemics.

325. The immunobiotic Clostridium butyricum S45-5 displays broad spectrum of antiviral activity in vitro and in vivo

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Clostridium butyricum is known as a probiotic butyric acid bacterium that can improve the intestinal environment. In this study, we isolated a new strain of C. butyricum from infant feces and evaluated its physiological characteristics and antiviral efficacy by modulating the innate immune responses in vitro and in vivo. The isolated C. butyricum S-45-5 (S-45-5) showed typical characteristics of C. butyricum including bile acid resistance, antibacterial ability, and growth promotion of various lactic acid bacteria. As an antiviral effect, S-45-5 markedly reduced the replication of influenza A virus, Newcastle Disease Virus, and Herpes Simplex Virus in RAW264.7 cells in vitro. This suppression can be explained by the induction of antiviral state in cells by the induction of antiviral, IFN-related genes and secretion of IFNs and pro-inflammatory cytokines. In vivo, oral administration of S-45-5 exhibited prophylactic effects on BALB/c mice against fatal doses of highly pathogenicmouse-adapted influenza A subtypes (H1N1, H3N2, and H9N2). Before challenge with influenza, S-45-5-treated BALB/c mice showed increased levels of IFN-b, IFN-g, IL-6, and IL-12 in serum, the small intestine, and bronchoalveolar lavage fluid, which correlated with observed

prophylactic effects. Interestingly, after challenge with influenza virus, S-45-5-treated BALB/c mice showed reduced levels of pro-inflammatory cytokines and relatively higher levels of anti-inflammatory cytokines at day 7 post-infection. Our study provides the beneficial effects of the new S-45-5 with antiviral effects as a probiotic. [The National Research Foundation of Korea (2021R1A6A1A03045495)]

326. Anti-Respiratory Syncytial Virus activity of Sargassum fusiforme extract and its components in vitro and in vivo

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Sargassum fusiforme, a plant used as a medicine and food, is regarded as a marine vegetable and health supplement to improve life expectancy. Here, we demonstrate that S. fusiforme extract (SFE) has antiviral effects against respiratory syncytial virus (RSV) in vitro and in vivo mouse model. Treatment of HEp2 cells with a non-cytotoxic concentration of SFE significantly reduced RSV replication, RSV-induced cell death, RSV gene transcription, RSV protein synthesis, and syncytium formation. Moreover, oral inoculation of SFE significantly improved RSV clearance from the lungs of BALB/c mice. Interestingly, the phenolic compounds eicosane, docosane, and tetracosane were identified as active components of SFE. Treatment with a non-cytotoxic concentration of these three components elicited similar antiviral effects against RSV infection as SFE in vitro. Together, these results suggest that SFE and its potential components are a promising natural antiviral agent candidate against RSV infection. [The National Research Foundation of Korea (2021R1A6A1A03045495)]

327. Extracts of Aster tataricus and its component display broad spectrum of antiviral activity in Vitro and in Vivo by inducing antiviral state

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Aster tataricus, a perennial terrestrial herb with a rich history in traditional medicine, is renowned for its therapeutic properties. Despite its widespread use, the antiviral activity against Influenza viruses remained unexplored. In here, we comprehensively assessed the antiviral activities and mechanisms of action associated with Aster tataricus extract (ASE) both in vitro and in vivo. The effective dose of ASE demonstrated marked inhibition of Influenza A virus, Newcastle disease virus, and Herpes simplex virus replication in immune cells (RAW264.7). This inhibition was attributed to the induction of an antiviral state through the upregulation of interferon (IFN)-related genes, coupled with the secretion of IFNs and pro-inflammatory cytokines. In vivo, experiments with ASE-treated BALB/c mice revealed enhanced survivability and reduced lung viral titers when challenged with lethal doses of highly pathogenic influenza A subtypes (H1N1, H5N2, and H9N2). The observed prophylactic effects correlated with increased secretion of IL-6, IFN-γ, and IFN-β in bronchoalveolar lavage fluid of treated mice. High-Performance Liquid Chromatography analysis identified several active compounds in the aqueous fraction, and subsequent evaluation highlighted the antiviral properties of Quercetin, Kaempferol, and Ferulic acid. This research establishes that ASE and its identified components function as immunomodulators, showcasing potential as broad-spectrum anti-viral and anti-influenza agents. The findings open avenues for further exploration of ASE in the development of therapeutic interventions against viral infections [National Research Foundation of Korea (2021R1A6A1A03045495)].

328. Anti-Influenza effects of Lactobacillus reuteri BSA218 by Modulating Innate Immunity

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The influenza virus causes severe respiratory infections worldwide. Vaccines and antiviral drugs have limited efficacy due to evolving virus resistance. Prophylactic therapies are necessary to boost host immune components. Probiotics, including lactobacillus species, provide health benefits and are naturally found in the human digestive and urinary tracts. This study investigated the probiotic function of Lactobacillus reuteri BSA218 against the influenza A virus (IVA) in both in vitro and in vivo settings. Pretreatment with BSA218 in RAW264.7 cells demonstrated upregulation of antiviral gene transcription, pro-inflammatory cytokines, phosphorylation of IFN-I-related proteins, and reduced replication of RNA and DNA viruses. Oral administration of BSA218 provided 100% protection against subsequent lethal influenza A infection, preventing significant weight loss and reducing lung viral loads in a C57BL/6NHsd mouse model. BSA218-treated mice exhibited elevated levels of cytokines and mRNA expressions of different antiviral molecules in serum, bronchoalveolar lavage fluids (BALFs), and small

intestinal fluids (SIFs), as well as a low degree of inflammation following influenza virus infection. Furthermore, we discovered that the bioactive lipids of BSA218 possess prophylactic potential against viruses. Collectively, these findings shed new light on the inhibitory effects of BSA218 on IVA replication, both in vitro and in vivo, and highlight its potential in probiotics-based antiviral research. [National Research Foundation of Korea (2021R1A6A1A03045495)]

329. Mesenchymal stem cells as immunomodulatory and regenerative agent for SARS-CoV-2 infection

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Background: Human mesenchymal stem cells (MSCs) are multipotent cells with an immunomodulatory, anti-viral and regenerative properties. We hypothesized that MSCs and conditioned medium (MSC-CMs) can dampen lung inflammation and repair lung tissue damage. The aim of study was to investigate the immunomodulatory and regenerative properties of MSCs and MSC-CMs on SARS-CoV-2 infection.

Materials and Methods: Calu-3, was used to model lung tissue air-liquid interface (ALI) in vitro. The model was infected with SARS-CoV-2 and co-cultured with MSCs or MSC-CMs (n=3) for 4 days. The impact of MSCs and MSC-CMs on Calu-3 was evaluated by measuring gene expression of inflammatory cytokines and immunostaining of epithelial integrity markers, and RNA-sequencing was analyzed.

Results: MSCs and MSC-CMs significantly reduced the inflammatory markers IL4, IL6, IL8, IL13,TNFα and increased the expression of epithelial integrity marker, CDH1 after infection. The disruption of lung epithelial tight junction was repaired by the MSCs and MSC-CMs. RNA-sequencing analysis showed immunomodulatory and regenerative pathways in the presence of MSCs and MSC-CMs.

Conclusion: MSCs and MSC-CMs can reverse the damage to lung epithelial cells caused by SARS-CoV-2 infection by inhibiting inflammatory cytokines and improving the integrity of epithelial cells. These findings support the potential for using MSCs or their by-products in cell therapy modalities.

330. Establishment of an integrated assay platform for supporting the discovery of antivirals and vaccines against human metapneumovirus infection

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HMPV is one of the main pathogens causing upper and lower respiratory tract infections in young children, older adults and immunocompromised patients. HPMV infection can lead to bronchiolitis, pneumonia, as well as acute asthma exacerbations. Currently, there is no vaccine or antiviral for the prevention and treatment of HMPV infection. To expedite the development of drugs and vaccines against HMPV infection, we have established an integrated platform including the conventional cell-based viral infection and neutralization assays, and a mouse model, as well as non-infectious replicon and cell fusion assays. In the mouse HPMV infection model, the efficacy of antivirals and vaccines can be evaluated with multiple endpoints including lung virus load and pathology. Our HMPV replicon contains all virus genes except for the envelope glycoproteins, and has a green fluorescent protein reporter. Since it is non-infectious and does not cause virus-induced cell-cell fusion, the replicon can be used for screening inhibitors acting on intracellular viral targets, such as the polymerase. Furthermore, the replicon can be applied to conduct the study of virus drug resistance, including de novo in vitro selections of drug resistance. HPMV inhibitors have been tested in the replicon and exhibited the comparable inhibitory activities to those observed in the cell-based HPMV infection assay. In summary, we have established and applied a panel of the in vitro and in vivo HPMV models which can facilitate the discovery of various types of antivirals and vaccines for the treatment and prevention of HPMV infection.

331. Evaluation of the Antiviral Activity and Cytokine Response of EIDD-1931 and Ensitrelvir in a specialized 3D normal, human tracheal/bronchial (EpiAirway) tissue model infected with SARS-CoV-2

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The EpiAirway tissue model provides a sensitive, human-derived cell culture assay to evaluate the antiviral activity of novel compounds. We have previously utilized these assay systems for the identification of active antiviral compounds against respiratory pathogens including Severe Acute Respiratory Syndrome-Associated Coronavirus 2 (SARS-CoV-2). We evaluated two compounds, EIDD-1931 a novel nucleoside analog which functions as a polymerase inhibitor and ensitrelvir which functions as an inhibitor of the SARS-CoV-2 3CL protease. The EpiAirway tissue cells were infected with the ancestral WA-1/2020 strain and the hCoV-19/USA/MDHP20874/2021 (B.1.529) omicron variant of SARS-CoV-2 and evaluated by virus yield reduction assay to determine a 90% effective concentration (EC90). The cytokine response of the EpiAirway model was evaluated using a multiplex assay for human cytokine and chemokine concentrations (Quansys Biosciences, Logan, UT). Virus titers in EpiAirway cells were reduced by treatment with EIDD-1931 with EC90 values of below 0.1 μM against both SARS-CoV-2 strains. EIDD-1931 also reduced virus titers with EC90 values of 0.26 and 0.18 μM against the ancestral and omicron variants respectively. The cytokine response was more affected by compound treatment in the EpiAirway cells infected with the omicron variant compared to the ancestral strain. Concentrations of Interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-) were significantly reduced in cells treated with doses of 3.2 and 1.0 μM EIDD-1931 and ensitrelvir. This effect was only statistically significant in the cells infected with the omicron variant.

332. Discovery and characterization of EGT710, an oral SARS-CoV-2 Mpro inhibitor and clinical candidate

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Antiviral therapies are a critical part of the healthcare response to SARS-CoV-2. Here we report the discovery and characterization of EGT710, an orally bioavailable coronavirus main protease inhibitor with potent in vitro activity against SARS-CoV-2 as well as other coronaviruses. EGT710 displays favorable pharmacokinetic properties and showed efficacy in a mouse model of SARS-CoV-2 infection. Modeling of human pharmacokinetics and viral kinetics demonstrated that EGT710 could reach concentrations predicted to be efficacious in humans. Following preclinical safety studies, EGT710 has now completed Phase I clinical trials.

333. Comparative Analysis of Resistance Mutation Emergence in SARS-CoV-2 Under Single and Combination Antiviral Therapies

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In the fight against SARS-CoV-2, both 3CL protease inhibitors, specifically nirmatrelvir (NTV) and ensitrelvir (ETV), and the nucleoside analogue molnupiravir (MPV), have been authorized for therapeutic use. While combination therapies, particularly NTV and MPV, have shown improved efficacy, their impact on the suppression of drug resistance remains unclear. Our study aimed to assess the frequency of drug resistance mutations in SARS-CoV-2 subjected to either single or combination antiviral therapies in vitro. We analyzed the emergence of resistance mutations in the virus under treatment regimens combining two inhibitors, compared to monotherapies with each drug, over five in vitro passages of SARS-CoV-2. The combination therapies significantly delayed viral growth compared to single-drug treatments, as measured by cytopathic effect analysis. In addition, comprehensive mutation analysis was performed using Next Generation Sequencing on all passaged viral samples, identifying 14 reported and 18 novel mutations within the 3CLprotease and RdRp genes. Contrary to expectations, combination therapies led to a more rapid induction of resistant mutations than monotherapies in vitro condition. Among the treatments, NTV was more effective in minimizing the emergence of mutations compared to ETV. As expected, MPV treatment resulted in the highest rate of synonymous and non-synonymous mutations throughout the passages. Our study suggests that while combination therapies are more efficacious in hindering SARS-CoV-2 growth, they may not effectively prevent the development of drug resistance.

334. From Barrier to Target: How Differentiation Impacts Upper Airway Epithelium Susceptibility to HMPV

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Human metapneumovirus (HMPV) is a major cause of upper respiratory tract infections, particularly in vulnerable populations. Despite its high incidence and continuous effort in therapeutics development, neither vaccine nor drug is currently available for the prevention or treatment of HMPV infection.

HMPV has the capacity to infect ciliated cells from the apical side of the airway epithelium. Propagation of HMPV is prolific only when primary epithelial cells remain in an "undifferentiated" state. A recent study using small airway organoid cultures confirmed the infection susceptibility of ciliated cells to HMPV. However, the HMPV initial infection site is the epithelium of the upper airway with possible migration to the small airway epithelium at a late stage of the infection. Our study reveals how HMPV infection rapidly declines once differentiation of primary upper airway epithelial cells at air-liquid interface is initiated, confirming the importance of basal cells for infection. By creating wounds in differentiated nasal epithelium, we restore a high level of infection at the wound site by mechanically exposing basal cells to the virus. Increased infection at the wound site contributes to promote infection to the entire differentiated nasal epithelium. This research provides a significant outcome in understanding the importance of upper respiratory epithelium integrity to prevent HMPV infection. Wounded nasal epithelium also offers an alternative model to in vivo models for early evaluation of drug candidates. It also questions the importance of co-infection with other viruses which could naturally expose basal cells to HMPV.

335. Intranasal Antivirals Against Respiratory Syncytial Virus: The Current Therapeutic Development Landscape

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Respiratory syncytial virus (RSV) causes bronchiolitis and other respiratory issues in immunocompromised individuals, the elderly, and children. After six decades of research, we have only recently seen the approval of two RSV vaccines, Arexvy and Abrysvo. Direct-acting antivirals against RSV have been more difficult to develop with ribavirin and palivizumab giving very modest reductions in hospitalisations and no differences in mortality. Recently, nirsevimab was licenced and has proven to be much more effective when given prophylactically. These are delivered intravenously but an intranasal antiviral has several advantages in terms of ease of use, lower resource need, and acting at the site of infection. In this poster, we review the available literature on the current pre-clinical research landscape of anti-RSV therapeutics tested for intranasal (IN) delivery. As RSV is a respiratory virus that infects both the upper and lower respiratory tracts, efforts are focused on developing a therapeutic that can be delivered via the nasal route. The rationale behind this is to directly target the replicating virus with an obvious respiratory tract tropism. This approach will not only pave the way for a nasal delivery approach aimed at reducing respiratory viral illness but also controlling aerosol virus transmission.

350V. Characterising mutational pressures exerted by nucleoside analogues on SARS-CoV-2 evolution

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Molnupiravir, an antiviral drug licensed to treat SARS-CoV-2 infection, acts by inducing mutations in the virus genome during replication. Most random mutations are likely deleterious, and many will be lethal; thus, molnupiravir-elevated mutation rates reduce viral load. However, if some patients treated with molnupiravir do not fully clear the SARS-CoV-2 infection, there is potential for onward transmission of molnupiravir-mutated viruses. We demonstrated that SARS-CoV-2 sequencing databases contain extensive evidence of molnupiravir mutagenesis. Using a systematic approach, we found that a specific class of long phylogenetic branches, distinguished by a high proportion of G-to-A and C-to-T mutations, are found almost exclusively in sequences from 2022, after the introduction of molnupiravir treatment and in countries and age groups with widespread use of the drug. We identified a mutational spectrum with preferred nucleotide contexts from viruses in patients treated with molnupiravir, revealing that its signature matches that seen in these long branches, in some cases with onward transmission of molnupiravir-derived lineages. In addition, we analysed treatment records to confirm a direct association between these high G-to-A branches and molnupiravir usage. Finally, in vitro experiments demonstrated sustained elevated G-to-A and C-to-T mutations 20 passages after initial treatment, suggesting treatment has long-lasting effects on viral populations if initial treatment is not lethal to the virus. Mechanistic characterisation of these genomic findings is critical to risk-assessing the consequences of the widespread use of mutagenic drugs for SARS-CoV-2 evolution.

351V. NEPTUNO: A Phase III Clinical Study of Plitidepsin for the Treatment of Adult Patients with COVID-19 Requiring Oxygen Therapy

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Plitidepsin (PLT) is a marine peptide with potent anti-SARS-CoV-2 and immunomodulatory preclinical activities. A phase I trial (APLICOV-PC, NCT04382066& NCT05121740) showed feasibility in patients (pts) with COVID-19. NEPTUNO was a phase 3, multicenter, randomized, controlled trial of PLT vs control in adult pts with COVID-19 (NCT04784559). Eligibility included: documented SARS-CoV-2 infection; <=14 days (d) from onset; <=3d corticoids; hospitalization; oxygen therapy (O2T); adequate organ function. Non-eligibility: severe physical dependency; chronic O2T; respiratory failure; severe COVID-19; concomitant antivirals, immunomodulatory or immunosuppressants. All pts received 3 d of dexamethasone (dex) IV plus either PLT (1.5 mg/d or 2.5 mg/d IV d1-3) or standard of care (SOC), followed by dex as clinically indicated. Primary endpoint: time to sustained withdrawal of O2T. A sample size of 609 pts (203/arm) and 530 events were needed to detect a target hazard ratio (HR) of 1.4 (1-sided type I error=1.25% (Bonferroni); power ≥ 80%). NEPTUNO prematurely ended on 31-Jan-23, with 205 randomized pts, due a significant accrual drop. There was a 2 d improvement in the median time to sustained O2T discontinuation (5 vs 7 days) favoring both PLT arms. For PLT 1.5 mg vs SOC, HR=1.37 (p=0.08), and for PLT 2.5 mg vs SOC, HR= 1.06 (p=0.78). A bootstrap simulation estimated HR=1.36 (adjusted p=0.0067) for PLT 1.5 mg vs SOC. Baseline IL-10 was a negative prognostic factor. Cox regression interaction model for IL-10 x PLT1.5mg and 2.5mg vs SOC estimated HR=1.78 (p=0.02) and HR=1.32 (p=0.27), respectively.

Data support a positive benefit-risk ratio for PLT in COVID-19.

352V. A Comprehensive Depiction of Events and Signaling Pathways Involving in SARS-CoV-2 Spike Protein-mediated Syncytia Formation

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The infection process of SARS-CoV-2 initiates with the binding of its spike protein (S) to the receptor-angiotensin-converting enzyme 2, leading to conformational changes in the S protein and fusion of viral and cellular membranes. Notably, the expression of S protein in infected pneumocytes can result in syncytia formation, contributing to severe COVID-19 progression. To quantitatively assess S protein-mediated cell-cell fusion, we established a syncytia formation assay using bimolecular split-Nanoluc complementation. Following a high throughput screening of ReFRAME and natural product libraries containing over 15,000 compounds, we identified multiple compounds inhibiting cell fusion. These hits exhibit pharmacological activities affecting various aspects of the viral life cycle and cellular signaling pathways, including Na+/K+ ATPase, androgen and estrogen receptor, neurotransmitter receptors, calcium fluxion, lipid metabolism, protein kinases, and cellular proteases. Among these, calcium fluxion appears to be a crucial node linking the various intracell signaling related to fusion. However, validation using SARS-CoV-2 pseudo virus revealed that most cell-cell fusion inhibitors did not block viral entry. In addition, some compounds exhibited potent activities in both inhibiting and enhancing cell fusion, in a cell type-dependent manner. In conclusion, our results contribute to a comprehensive understanding of events and signaling pathways involved in the S-mediated cell fusion. These findings emphasize notable distinctions in the factors associated with virus-cell fusion and cell-cell fusion, shedding light on potential drugs for mitigating the progression of COVID-19.

353V. Predictive assessment of Imperata cylindrica antiviral properties against SARS CoV-2

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The Omicron variant and its sublineages are highly contagious and they still constitute a global source of concern. Despite vaccinations, hospitalizations and mortality rates resulting from infections by these variants of concern the existing therapeutic alternatives have presented various setbacks such as low potency, poor pharmacokinetic profiles and drug resistance. The need for effective therapeutic alternatives cannot be overemphasized. Plants and their phytochemicals present interesting characteristics that make them suitable candidates for the development of antiviral therapeutic agents. This study aimed to investigate the inhibitory effects of Imperata cylindrica (I. cylindrica) phytochemicals against SARS-CoV-2 main protease (Mpro), a highly conserved protein among coronaviruses.

Molecular docking and in-silico pharmacokinetic assays were used to assess 72 phytocompounds that are found in I. cylindrica as ligands and Mpro (6LU7) as the target. Only eight phytochemicals (bifendate, cylindrene, tabanone, siderin, 5-hydroxy-2-[2-(2-hydroxyphenyl)ethyl]-4H-1-benzopyran-4-one, maritimin, 5-methoxyflavone and flavone) displayed high binding affinities with Mpro with docking scores ranging from -5.6 kcal/mol to -9.1 kcal/mol. The in-silico pharmacokinetic and toxicological assays revealed that tabanone was the best and safest phytochemical for the development of an inhibitory agent against coronavirus main protease. Thus, the study served as a baseline for further in vitro and in vivo assessment of the phytochemical against Mpro of SARS CoV-2 variants of concern to validate the in-silico findings.

354V. 6-Azauridine Prodrugs as Anti-Influenza Inhibitors

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Influenza virus is a high-risk virus that causes 3-5 million severe cases annually worldwide. The Influenza A virus (IAV) is the most threatening virus among the four antigenic types of influenza viruses (Influenza A, B, C and D), and has caused four pandemics in the twentieth century, the latest being in 2009. Currently, the approved treatments for Influenza suffer from drug resistance issues, indicating a critical need for development of novel antiviral agents capable of targeting this virus. We developed a high throughput screen against Influenza virus and screened 200K unique compounds, from which 6-Azauridine (6-AzaU) was identified and then reconfirmed in the antiviral and virus titer reduction (VTR) assays. 6-AzaU showed antiviral inhibition against IAV H1N1 and H3N2 (EC90 = 1.1 μM and EC90 = 1.3 μM, respectively) with no cytotoxicity up to 20 μM. We adopted a nucleoside prodrug approach to improve the bioavailability and cell permeability of 6-AzaU. These efforts led to the identification of SRI-44472, which showed similar H1N1 and H3N2 potency and improved ADME and PK properties. SRI-44472 also showed inhibition of U2-PB2 chimeric RNA synthesis in a Cap-snatch polymerase assay (EC50 = 2.1 μM). The structure activity relationship (SAR) studies and biological results, including plasma protein binding and in vivo data of SRI-44472, will be discussed.

355V. Exploiting DKA derivatives as SARS-CoV-2 nsp13 inhibitors active on viral replication

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The outbreak of the novel coronavirus SARS-CoV-2 in late 2019 has presented an unprecedented global health challenge and highlighted the risk of a zoonotic spillover into human population, bringing the attention on the development of coronaviruses antivirals. Currently, some small-molecule antivirals targeting two viral proteins, Mpro and RdRp, have been approved for therapeutic use in some countries and regions worldwide. Considering the crucial role of RNA helicases in viral replication cycles and the high sequence identity among all known coronaviruses, SARS-CoV-2 nsp13 represents a validated target for drug discovery. Nsp13 is a multidomain enzyme able to unwind DNA or RNA in an NTP-dependent manner with a 5'-3' polarity. It couples two C-terminal RecA ATPase domains, characteristic of the 1B (SF1B) helicase superfamily, with other three domains: the N-terminal zinc-binding domain (ZBD), essential for the helicase activity, a stalk, and a 1B domain. In the present study, we exploit the new class of indolyldiketoacids (DKA) derivatives recently identified as nsp13 inhibitors [1], starting from the optimization of our previous DKA scaffolds exhibiting broad-spectrum antiviral activity. The class of 88 new compounds was tested on both the SARS-CoV-2 nsp13 unwinding and ATPase associated activities. Among them, 8 compounds inhibit both nsp13 enzymatic functions in a significant low micromolar range and were selected for the evaluation of their activity in blocking SARS-CoV-2 replication. DKAs showed the capacity to inhibit viral replication of SARS-CoV-2 providing strong rationale to evaluate potential broad-spectrum antiviral activity.

[1]Corona et al 2023 AVR

356V. Travatrelvir, a Potent Inhibitor of SARS-CoV-2 Main Protease now in Phase 1 Clinical Trials, Shows a Superior Drug Resistance Profile in vitro Compared to Nirmatrelvir

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Inhibitors of the SARS-CoV-2 main protease (Mpro/3CL) provide valuable antiviral therapy options for persons with COVID19. Nirmatrelvir is the only approved Mpro inhibitor in the U.S. and Europe, but it requires coadministration of the CYP450 inhibitor ritonavir. Our novel Mpro inhibitor travatrelvir, developed with assistance from the Expert Systems accelerator, is more potent than nirmatrelvir and, in 5-day preclinical studies, did not require ritonavir co-administration. Here, we report the patterns of resistance to travatrelvir that were observed during in vitro selection for drug-resistant variants, using travatrelvir at 400 nM (100xEC50). There was extensive overlap between the drug resistance variants reported for nirmatrelvir (Iketani, et al, Nature 613:558, 2023) and travatrelvir. However, two major paths to nirmatrelvir resistance, involving P252L and T304I, were not observed after travatrelvir selection. Travatrelvir (EC50 4 nM) was more potent than nirmatrelvir (IC50 27 nM) in inhibiting the activity of wild type Mpro and was also more potent than nirmatrelvir against Mpro with the S144E, E166V or A173V mutations. The T21I mutation, which represents less than half of the nirmatrelvir resistance mutations, was present in 68% of travatrelvir-resistant variants, but the P252L and T304I mutations, comprising a large portion of nirmatrelvir resistance, were not detected in our study. The patterns of resistance to travatrelvir overlap with reported resistance mutations for nirmatrelvir but important differences were observed. Travatrelvir has an overall advantage in potency and may be preferred if P252L or T304I variants are present in the circulating virus pool.

400V. Identification of the new HSV-2 variant (HSV-2v) in two patients suffering from genital herpes not responding to valacyclovir therapy

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HSV-1 and HSV-2 are prevalent human pathogens (global seroprevalence of 66% and 13.2%, respectively), establishing long-life latency in neurons and reactivating periodically. HSV-1 preferentially infects oral mucosa while HSV-2 more often causes genital herpes, though both viruses can infect either mucosa. As all herpesviruses, they are considered to exhibit low genetic diversity [mean pairwise distance of 0.8% (HSV-1) and 0.1% (HSV-2)]. In the framework of our translational research platform RegaVir for diagnosing herpesvirus drug-resistance, 3 HSV-2 isolates [RV-938 (Patient #1); RV-2760 & RV-2765 (Patient #2)] were recovered from 2 non-immunocompromised Belgium women suffering from recurrent genital herpes under valacyclovir therapy for years. The isolates harbored no known thymidine kinase (TK) or DNA polymerase (DP) drug-resistance mutations but >20 novel changes in the DP, primarily in the thumb domain, when aligned to HSV-2 laboratory strains. Two novel TK changes (P273L & E276K) were also found in the RV-2760 and RV-2765 isolates. All these changes did not affect the sensitivity to anti-HSV-2 drugs, indicating their association with inter-strain variability. Alignment of the patients' DP sequences to different clinical HSV-2 isolates showed a high similarity to the new HSV-2 variant (HSV-2v) identified in sub-Saharan African patients, characterized by an unexpectedly high DP variability (2.4% divergence). We also found the HSV-2v variant in a man suffering from persistent HSV-2 ocular infections. To our knowledge, these are the first HSV-2v strains isolated in Belgium and their replicative fitness is being investigated in dual infection competition assays.

401. Helicase-primase inhibitor IM-250 efficiently controls herpes infections and recurrent disease by reducing the latent viral reservoir in animal models and shows sufficient exposure in a Phase 1 clinical trial

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Herpes is a contagious life-long infection with persistently high incidence and prevalence, causing significant disease worldwide. Current therapies have efficacy against active herpes simplex virus (HSV) infections but no impact on the latent viral reservoir in neurons. Thus, despite treatment, disease recurs from latency and the infectious potential remains unaffected within patients. In cell culture the helicase-primase drug IM-250 inhibits HSV replication including acyclovir-resistant strains with an IC50 in the range of 3 to 30 nM. IM-250 therapy effectively controlled viral disease in animal models of HSV infection. Treatment of lethally infected HSV-1 mice increases survival at an ED of 0.5 mg/kg. In the mouse HSV-1 ocular infection model 4 cycles of intermittent therapy with IM-250 starting 45 days post infection reduced the reactivation competence of the latent viral reservoir compared to no therapy. In the guinea pig HSV-2 vaginal infection model where animals develop spontaneous recurrences, IM-250 completely eliminated recurrences after 7 cycles of treatment while acyclovir and vehicle groups continued to develop recurrent disease over 6 months. We provide evidence in 2 animal models that antiviral treatment during HSV latency can reduce future reactivation from the latent reservoir, supporting a conceptual shift in the antiviral field, and reframing what is achievable with respect to therapy of latent neuronal HSV infections. A Phase 1 clinical trial in healthy volunteers shows sufficient exposure in human plasma without dose limiting toxicity. Phase 2 clinical trials in HSV patients are in preparation.

402. HIV-1 Topoisomerase IIβ kinase as novel target to inhibit viral reverse transcription

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Human Immunodeficiency Virus (HIV-1) upon infection enters CD4-positive immune cells hijacking and destroying the immune system; thus, culminating in a plethora of opportunistic infections leading to AIDS. HIV-1 infection progression depends on the successful overriding of cellular replication machinery for the pivotal reverse transcription step in viral replication. Our studies have shown the expression of a 72KDa protein in HIV-1 infected cells along with activation of cellular TopoisomeraseIIβ. Our investigations showed that the 72kDa protein (TopoIIβkinase) phosphorylates topoisomeraseIIβ upon infection. This exposed a novel target to inhibit infection progression and develop next-generation inhibitors. Our current study focuses on characterizing TopoIIβkinase and its inhibitors. We have developed three potent inhibitors from a series of dicoumarols structurally mimicking NNRTIs. These molecules showed very low cytotoxicity and high antiviral effects (IC50 ~40nMs). They are observed to inhibit viral replication and block RT at an early stage. Simultaneously novel delivery vehicles were developed using Lactoferrin for delivering into infected sites and organs. These nanoparticles were~250nm in diameter and showed an improved efficacy.

403. Discovery of Novel Aryl Triazolone Dihydropyridines (ATDPs) Targeting Highly Conserved Residue W229 as Promising HIV-1 NNRTIs

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Human immunodeficiency virus-1 (HIV-1) non-nucleoside reverse transcriptase inhibitors (NNRTIs) are an important component of the highly active antiretroviral therapy (HAART), but the rapid emergence of drug resistance and poor pharmacokinetics

limited their clinical application. In this work, a series of novel aryl triazolone dihydropyridines (ATDPs) was designed by enhancing the hydrophobic interactions with the highly conserved residue W229 in the NNRTIs binding pocket, with the aim to improve anti-HIV-1 potency and pharmacokinetics profiles. Compound 10n (EC50 = 0.009 – 17.7 μM) exhibited the most active potency, being superior to or comparable to that of doravirine (DOR) against the whole tested viral panel. The molecular docking was performed to clarify the reason for its higher resistance profiles. Moreover, 10n was demonstrated with excellent pharmacokinetics profiles with significantly improved oral bioavailability (F = 108.96%) and more favorable half-life (T1/2 = 5.09 h) compared that of DOR (F = 57%, T1/2 = 4.4 h). Additionally, 10n was also verified no in vivo acute or subacute toxicity (LD50 > 2000 mg/kg), suggesting that 10n can be used as a promising oral candidate for HIV-1 therapy.

404. Discovery of novel phenylalanine derivatives as potent HIV capsid modulators with improved antiretroviral activity and metabolic stability

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The HIV capsid (CA) protein is a promising target for anti-AIDS therapy due to its critical involvement in viral replication. In this study, we utilized the well-documented CA modulator PF74 as our lead compound, and designed a series of phenylalanine derivatives by specifically targeting the NTD-CTD interface within the CA hexamer. Among them, compound 7t exhibited remarkable antiviral activity with a high selection index (EC50 = 0.040 μM, SI = 2815), surpassing that of PF74 (EC50 = 0.50 μM, SI = 258). Furthermore, when evaluated against the HIV-2 strain, 7t (EC50 = 0.13 μM) demonstrated approximately fourteen-fold higher potency than PF74 (EC50 = 1.76 μM). An SPR-based evaluation highlighted the superior affinity of 7t for the HIV-1 CA hexamer and monomer compared to PF74. Additionally, the competition assay indicated that these compounds possess the ability to bind to the same pocket in CA hexamer as PF74. More in-depth molecular docking and MD simulation studies provided insights into the potential molecular interactions between 7t and the NTD-CTD interface, offering plausible explanations for the improved target affinity compared to PF74. The single-round infection (SRI) assay revealed 7t has a strong early-stage effect and a slightly lower late-stage effect (yet still significant compared to PF74). Notably, 7t displays superior metabolic stability (T1/2 = 77.0 min) in HLM, representing an impressive 110-fold improvement over PF74 (T1/2 = 0.7 min). Based on these findings, it can be concluded that compound 7t exhibits enhanced antiretroviral activity and remarkable metabolic stability, positioning it as a potential lead compound for further investigation.

500. Modeling the impact of antiviral therapy on liver disease associated with chronic hepacivirus infection

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The majority of humans infected with hepatitis C virus (HCV) become chronically infected, which if untreated can cause liver fibrosis, cirrhosis, and/or hepatocellular carcinoma (HCC). While curative antiviral therapy is available, successful cure may not prevent the development of severe liver disease. The underlying biology guiding the development of fibrosis and HCC resulting from chronic HCV infection remains unknown and there are few tractable animal models within which to dissect these mechanisms. We developed a mouse model of chronic HCV pathogenesis using a rodent homolog called Norway rat hepacivirus (NrHV) (2023 Brown et al. Hepatology) in the Collaborative Cross (CC), a mouse reference population with genetic diversity like that in the human population. We identified CC mouse lines that support chronic infection and develop liver

fibrosis and very recently have mapped the genetic loci associated with chronic infection. These chronic models can be used to elucidate the virus and host interactions driving viral pathogenesis and to determine when antiviral therapy fails to stop disease progression. To this end, we determined that chronic NrHV infection can be cured with molnupiravir. Current studies are aimed at varying the time of curative antiviral therapy to better understand the time at which therapy fails to prevent fibrosis. These studies should not only elucidate basic biological insights into liver biology and the interplay of the innate and adaptive immune responses that contribute to liver disease but also provide practical insights into the timing of antiviral therapy to maximize the prevention of severe liver disease.

501. Human Intestinal Enteroids as a new in vitro model for Hepatitis E Virus antiviral development

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Hepatitis E virus (HEV) is an emerging pathogen responsible for acute viral hepatitis globally. There is no specific and effective antiviral or vaccine, partly due to limitations in in vitro cultivation. Although HEV is mainly fecal-oral transmitted and excreted in the feces in high titers, the role of the gut in HEV-induced disease remained unexplored. Little is known about the virus spread from gut to liver, nor about the gut acting as an HEV reservoir. To tackle this, we developed an HEV infection model using Human Intestinal Enteroids (HIEs). HEV infection was done using: a) differentiated 3D-HIEs; b) differentiated 2D-HIEs in transwells; c) electroporation of HIEs with full-length HEV RNA or luciferase (luc) subgenomic RNA. 3D infection with HEV gt3 Kernow-C1 p6 (HEV-p6) or p6-G1634R (HEV-p6-R), harboring a fitness-enhancing mutation, resulted in a 1 log10 increase in HEV RNA up to 7 days post-infection (dpi) with replication peaking at 1 dpi. Infection of 2D-HIEs yielded similar replication levels, with shedding mainly to the apical side of the intestinal epithelial layer. Importantly, electroporation of HIEs with HEV-p6 or HEV-p6-R resulted in a sustained increase in viral load over time, reaching 3-5x105 vRNA copies/mL at 11 days post electroporation. Likewise, electroporation of HEV-p6-luc resulted in a 20-fold increase in luciferase signal. Infection was confirmed by immunostainings. The reference antivirals ribavirin, sofosbuvir and interferon-alfa efficiently blocked viral replication over time. We here successfully established an HEV cultivation model in HIEs suitable for antiviral screening, with electroporation being the most efficient.

502. Propiophenone Thiosemicarbazones as New Agents Against Bovine Viral Diarrhea Virus

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Antiviral activity of compounds containing the thiosemicarbazone (TSC) group has been described in previous studies. In some cases, TSCs derived from 1-indanone have been shown to act as non-nucleoside inhibitors (NNI) of the RNA polymerase of bovine viral diarrhea virus (BVDV). This study aimed to assess the antiviral potential and selectivity of 20 newly synthesized TSCs derived from benzaldehyde, acetophenone, and propiophenone against BVDV. Cytotoxicity (CC50) was evaluated in MDBK cells, and antiviral activity (CE50) against BVDV-1a was determined by viral cytopathic effect (CPE) reduction assays using MTS/PMS. The influence of different chemical groups on antiviral activity was explored by SAR and cross-resistance against a known BVDV RNA polymerase inhibitor was evaluated for the most active TSCs.

Most TSCs exhibited low cytotoxicity (CC50 > 200 μM). Propiophenone derivatives displayed the highest antiviral activity (CE50 0.5-5 μM), in comparision to acetophenone (5-10 μM) and benzaldehyde derivatives (>10 μM). In addition, propiophenone derivatives with electron-withdrawing groups in the R3 position showed the highest efficacy, highlighting the importance of these groups at this position. Notably, one of the two most active TSCs displayed cross-resistance to a BVDV mutant resistant to a TSC derived of 1-indanone, while the other remained susceptible, indicating potential for further investigation. In conclusion, this study identified a promising TSC derived of propiophenone with potent antiviral activity against BVDV a no cross-resistance with another non-nucleoside inhibitor of BVDV, which highlights its potential for further development as a novel antiviral.

503. Development and establishment of reporter replicons for antiviral drug discovery against human Noroviruses

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Human noroviruses (HuNoV) are recognized as the predominant cause of viral gastroenteritis globally, posing a substantial socio-economic burden across diverse geographical regions. However, the ongoing challenges in culturing these viruses have been a major barrier to the development of targeted therapies and in-depth knowledge of the cellular processes that control virus infection. Advances in reverse genetics have catalyzed the emergence of reporter replicon systems as a valuable alternative for the exploration and testing of direct-acting antivirals, as well as for uncovering crucial host factors that are pivotal during the viral life cycle. Here, we develop and establish HuNoV reporter replicon systems allowing us to investigate both GI and GII genotypes. We summarize the strategies used to develop the system ensuring their robustness and reliability through rigorous validation processes involving well-characterized norovirus inhibitors. Our findings demonstrate the efficacy of reporter replicons as a dependable platform for the systematic screening and identification of compounds capable of inhibiting viral replication. The replicon systems described provide additional tools in the ongoing pursuit to discover direct-acting antivirals against human noroviruses.

504. Antiviral Inhibition of Human Norovirus ProPol Precursor

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Human norovirus (HuNV) is a leading cause of acute gastroenteritis in all age groups but there are no approved vaccines or antiviral therapies available. Development of HuNV antivirals has primarily targeted mature protease and polymerase (Pol) enzymes. However, viral replication generates several precursor proteins, including ProPol, the protease-polymerase precursor that is functionally active and important for nucleotidylylation of the viral VPg. In this study we have investigated the effect of direct-acting antivirals on polymerase activity of genogroup I (GI) and genogroup II (GII) strains of the HuNV ProPol precursor. Dose-response curves of ProPol with the nucleoside analogue galidesivir triphosphate (Gal-TP), or the non-nucleoside analogue PPNDS were generated to measure polymerase inhibition. IC50 values of 247.5 μM with Gal-TP and 3.8 μM with PPNDS were obtained, showing the antivirals inhibit ProPol activity. In both instances, but particularly with PPNDS, the ProPol IC50 values were greater than those with mature Pol indicating that the inhibitors were more effective against Pol. We also investigated whether inhibition of protease activity can influence polymerase activity of ProPol using NV-004, a peptidomimetic protease inhibitor. Incubation with 30 μM NV-004 decreased polymerase activity by 38% and 24% for GI and GII ProPol respectively. NV-004 had no effect on polymerase activity of mature Pol from either HuNV strain. The results with Gal-TP, PPNDS and NV-004 indicate that HuNV ProPol is an important antiviral target with unique susceptibilities compared to mature Pol.

505. Exploring Antiviral Candidates for Human Norovirus: Utilizing the Pandemic Response Box's Open-Source Compound Repository

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The Pandemic Response Box initiative, collated and distributed by the Medicine for Malaria Venture, has enabled a global collaborative effort by distributing a collection of 400 diverse compounds to research entities worldwide, aiming to accelerate the discovery of treatments for pandemics and infectious diseases. Our project participates in this initiative by evaluating these compounds for their potential to combat norovirus, the leading cause of acute gastroenteritis globally. Although human norovirus (HuNoV) imposes significant health and economic impacts, no vaccines or treatments have been approved so far. To investigate the antiviral properties of these compounds, we have implemented two orthogonal screening assays. One employs live murine norovirus (MNV) infection in murine microglial cells (BV2), while the other uses a human norovirus replicon system in human gastric cells (HGT). We monitor the impact of these compounds on MNV infection using an Incucyte real-time cell imaging based approach. The effect on human norovirus replication is assessed examining the impact of compounds on viral RNA levels in HGT cells carrying a Norwalk virus replicon using a highly scalable, extraction free, RT-qPCR assay. These assays have demonstrated robust assay metrics and have been validated using known compounds with anti-norovirus activity. The outcomes of this research are expected to be instrumental in leveraging our collective expertise in pathogen biology towards identifying new starting points for drug discovery.

506. PDZ Interactions as Determinants of Viral Pathogenicity and Therapeutic Targets

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Interactions between host PDZ-containing proteins and viral PDZ-binding motifs (PBMs) are pivotal in the pathogenicity of various viruses, including Rabies virus (RABV), Influenza A virus, and SARS-CoV. Viruses exploit PDZ domains, essential for complex assembly and cellular regulation, to subvert host processes for replication and survival. RABV specifically targets the PDZ domain of neuronal enzymes, disrupting cellular enzyme complexes and signaling pathways, influencing the fate of infected cells. Influenza A virus interacts with PDZ-containing proteins, affecting viral assembly and release. These viruses possess PBMs in their proteins, enabling interactions with host PDZ-containing proteins. This manipulation allows viruses to manipulate cellular machinery, evade immune responses, and promote their replication. Hepatitis B virus (HBV) mimics cellular PDZ-binding motifs to exploit the PDZ domain of host proteins, causing disruptions in cellular complexes and signaling. Through various biophysical, structural, and biological evidence, we showed that the HBV capsid may interact with specific PDZ proteins in host cells. These interactions potentially play a pivotal role in the virus's life cycle, influencing processes such as viral entry, exit, and the regulation of viral replication. Understanding these interactions provides insights into viral pathogenicity mechanisms, offering opportunities for antiviral strategy development. Inhibitory compounds disrupting viral PDZ-binding motif interactions with host PDZ-containing proteins could be a promising therapeutic approach, blocking key viral life cycle aspects and providing innovative strategies against infections.

507. Assay development to test viral intrinsically disordered regions as potential antiviral targets

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Intrinsically disordered proteins regions (IDRs) of proteins are stretches of amino acids that lack structure but maintain function. Viral proteins are often enriched with IDRs and their interactions with other proteins can be dynamic, low affinity and transient. This could allow a single viral protein to bind to multiple viral or cellular partners and influence viral replication in a variety of ways. This multifunctional nature of viral IDRs makes them potential antiviral targets and their inhibition could create a multi-

layered offensive during viral infection. However, low affinity interactions can be difficult to measure. To combat this a sensitive NanoBit protein:protein interaction assay was developed. The interaction between the disordered N-terminal region of SARS-CoV-2 nucleocapsid or human norovirus NS1-2 proteins and human cyclophilin A was utilised to test lead compounds identified from a compound library.

508. Development of Novel Anti-HBV Agents: Characterization of the N-Hydroxypyridinediones (HPDs) as HBV RNase H Inhibitors

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HBV is a DNA virus in the Hepadnaviridae family. Long-term HBV infections constitute a major cause of end-stage liver disease, cirrhosis, liver failure and hepatocellular carcinoma. Current antiviral therapy rarely eradicates the virus and cleared HBV infections can be reactivated. Moreover, HBV's high mutation rate can lead to drug resistance. To cure HBV infection, it is crucial to develop new strategies. HBV ribonuclease H (RNaseH) is a metalloenzyme (nucleotidyl transferase superfamily) and its active site contains four carboxylates that bind to two Mg2+ ions required for RNA cleavage. However, the potential of RNaseH as a drug target for HBV treatment was not explored until recently. The importance of the RNaseH and its low amino acid homology with the cellular RNaseHs, prompted the development of novel scaffolds, bearing a metal-chelating motif, as potent inhibitors. N-Hydroxypyridinediones (HPDs) are compounds that inhibit metalloenzymes. They are effective against the HBV RNaseH, suppressing HBV replication at sub-micromolar concentrations. Previously, the best compounds from these classes had selectivity indexes (SIs) of ~350 (HPD). We describe the synthesis, anti-HBV activity, cytotoxicity, pharmacological properties, and off-target effects of novel HPDs. All compounds tested had CC50s >100 μM in PHHs. Additionally, compounds had a satisfying t1/2 > 4 hr. Moreover, the HPDs were soluble and passively permeable at all pHs, and significantly effective at suppressing plus polarity strand synthesis – two had EC50 values of 90 and 60 nM, resulting in SIs of 1063 and 1120. These results indicate that the HPDs hold significant potential for antiviral development.

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