ICAR2023

PROGRAM & ABSTRACTS

13-17 MARCH 2023

Lyon, France

LYON CONVENTION CENTRE

36TH 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 36th year of existence, has members representing 30 countries. To become an ISAR member, visit our website at **www.isar-icar.com**.

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Confirmed sponsors as of March 7, 2023

PREMIER PLATINUM

GILEAD *
Creating Possible

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MSD INVENTING FOR LIFE

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

Virtual Platform Information

ICAR2023

ICAR2023 will offer both in-person and virtual programming options. Please find below details to assist you with navigating the ICAR2023 virtual platform.

>> How do I access the virtual platform?

All registered attendees (onsite and virtual) will receive log-in details on Monday, March 13. The ICAR2023 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, March 13, 2023.

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

All registered attendees will be able to access on-demand content through April 17, 2023.

>> 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 Roundtable 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. Click on the "Posters" tab, to view 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, ask to be contacted for more information, or even leave a simple 'hello' to check in, so that they know you visited. There will be no live Q&A for virtual posters.

>> 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 ICAR2023 to help you become familiar with the virtual platform.

Women in Science Roundtable Discussion

Monday, March 13 • 12:00 – 1:45 PM CET

ROSERAIE 1-2 (3rd Floor)

The WIS Committee is excited to hold the 11th Annual Women in Science Roundtable at the ICAR2023. Please join us to network with fellow scientists in industry, government, and academia who conduct all aspects of antiviral research. This session will provide an opportunity to participate in an exciting exchange of ideas with a panel of expert antiviral research scientists as well as other event participants.

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

Please note that while this event is open to everyone, registration is required as space is limited.

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

Opening Session and Plenary Speakers

Monday, March 13 • 2:00 – 4:15 PM CET

AUDITORIUM LUMIÈRE

- **Esteban Domingo**
  Spanish Research Council (CSIC)
  The Quasispecies Challenge: In Search of Antiviral Synergisms with Lethal Mutagens

- **Laurent Fraisse**
  Drugs for Neglected Diseases initative (DNDi)
  Addressing Viral Infections in Neglected Patient Populations: DNDi Efforts

Opening Reception

Monday, March 13 • 5:30 – 6:30 PM CET

FORUM 1

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 **ICAR2023**!

PeckaKucha Competition

Tuesday, March 14 • 11:00 AM – 12:00 PM CET
AUDITORIUM LUMIÈRE

Get ready to be entertained and informed as finalists present their PechaKucha presentations. Not famililar 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 Roundtable

Tuesday, March 14 • 12:00 – 1:30 PM CET
ROSERAIE 1-2 (3rd Floor)

This year, the ICAR career development session will be an interactive Career Roundtable. 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.

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

Late-breaking Oral Presentations

Wednesday, March 14
11:30 AM – 12:15 PM CET
AUDITORIUM LUMIÈRE

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

Closing Event

Thursday, March 16
6:00 – 8:00 PM CET
FORUM 2

Join us for an evening of networking with fellow attendees. 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 Central European Time (CET).

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The in-person sessions in Lyon 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.

Monday, March 13, 2023

TIME *(CET)*	EVENT	LOCATION
12:00 PM – 1:45 PM	**Special Event: Women in Science Roundtable***	ROSERAIE 1-2 (3rd floor)
2:00 PM – 4:15 PM	**Opening Session and Plenary Speakers**	AUDITORIUM LUMIÈRE
4:15 PM – 4:30 PM	**Break**	FORUM 1
4:30 PM – 5:30 PM	**Gertrude Elion Memorial Award Lecture**	AUDITORIUM LUMIÈRE
5:30 PM – 6:30 PM	**Opening Reception***	FORUM 1

Tuesday, March 14, 2023

TIME *(CET)*	EVENT	LOCATION
8:30 AM – 9:15 AM	**William Prusoff Memorial Award Lecture**	AUDITORIUM LUMIÈRE
9:15 AM – 10:15 AM	**SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals I**	AUDITORIUM LUMIÈRE
10:15 AM – 10:30 AM	**Break**	FORUM 1
10:30 AM – 11:00 AM	**SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals I** *(continued)*	AUDITORIUM LUMIÈRE
11:00 AM – 12:00 PM	**PechaKucha Competition**	AUDITORIUM LUMIÈRE
12:00 PM – 1:30 PM	**Special Event: Career Development Interactive Roundtable***	ROSERAIE 1-2 (3rd floor)
2:00 PM – 2:45 PM	**Women in Science Award Lecture**	AUDITORIUM LUMIÈRE
2:45 PM – 3:45 PM	**Chronic, Persistent, or Latent Viruses I**	AUDITORIUM LUMIÈRE
3:45 PM – 4:00 PM	**Break**	FORUM 1
4:00 PM – 5:00 PM	**Chronic, Persistent, or Latent Viruses I** *(continued)*	AUDITORIUM LUMIÈRE
5:00 PM – 7:00 PM	**Poster Session 1*** *(Light food and beverages provided)*	FORUM 1

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

Wednesday, March 15, 2023

TIME *(CET)*	EVENT	LOCATION
8:15 AM – 8:30 AM	**Tribute to Mike Bray**	AUDITORIUM LUMIÈRE
8:30 AM – 10:00 AM	**Influenza, RSV, and Other Respiratory Viruses**	AUDITORIUM LUMIÈRE
10:00 AM – 10:15 AM	**Break**	FORUM 1
10:15 AM – 11:30 AM	**Influenza, RSV, and Other Respiratory Viruses** *(continued)*	AUDITORIUM LUMIÈRE
11:30 AM – 12:15 PM	**Late-breaking Oral Presentations**	AUDITORIUM LUMIÈRE
12:15 PM – 2:15 PM	**Poster Session 2*** *(Lunch provided)*	FORUM 1

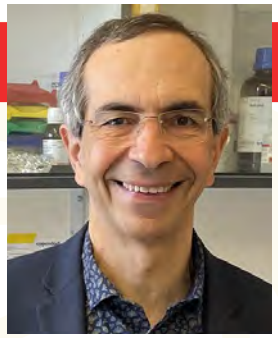
Thursday, March 16, 2023

TIME *(CET)*	EVENT	LOCATION
8:30 AM – 9:15 AM	**Antonín Holý Memorial Award Lecture**	AUDITORIUM LUMIÈRE
9:15 AM – 10:15 AM	**Chronic, Persistent, or Latent Viruses II**	AUDITORIUM LUMIÈRE
10:15 AM – 10:30 AM	**Break**	FORUM 1
10:30 AM – 12:00 PM	**Chronic, Persistent, or Latent Viruses II** *(continued)*	AUDITORIUM LUMIÈRE
12:00 PM – 12:15 PM	**ISAR Annual Business Meeting**	AUDITORIUM LUMIÈRE
2:00 PM – 2:45 PM	**Diversity Speaker Award Lecture**	AUDITORIUM LUMIÈRE
2:45 PM – 3:45 PM	**SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals II**	AUDITORIUM LUMIÈRE
3:45 PM – 4:00 PM	**Break**	FORUM 1
4:00 PM – 6:00 PM	**SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals II** *(continued)*	AUDITORIUM LUMIÈRE
6:00 PM – 8:00 PM	**Closing Event***	FORUM 2

Friday, March 17, 2023

TIME *(CET)*	EVENT	LOCATION
9:00 AM – 10:30 AM	**Acute GI Viruses**	AUDITORIUM LUMIÈRE
10:30 AM – 10:45 AM	**Break**	FORUM 1
10:45 AM – 12:00 PM	**Chronic, Persistent, or Latent Viruses III**	AUDITORIUM LUMIÈRE
12:00 PM – 12:30 PM	**Shotgun Presentations and Closing Remarks**	AUDITORIUM LUMIÈRE

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



GERTRUDE ELION MEMORIAL AWARDEE

Fabien Zoulim, PhD

Discovery Science for the Cure of Hepatitis B Virus Infection

Fabien Zoulim obtained his MD in Gastroenterology and Hepatology at the Lyon Medical School. He has also obtained a PhD in Molecular and Cellular Biology from Paris University and was trained as a post-doctoral researcher at Fox Chase Cancer Center in Philadelphia. He is Professor of Medicine at **Lyon I University** since 1997. He is Head of the Hepatology Department at the Hospices Civils de Lyon, and Head of the Viral Hepatitis Research Laboratory of **INSERM Unit 1052**. Dr. Zoulim is currently Associate Editor for GUT. He also served as a Governing Board member of the European Association for the Study of the Liver (EASL). His main research interest is the understanding of hepatitis B virus (HBV) persistence and the identification of novel mechanisms to cure HBV infection. Dr Zoulim received the William Prusoff award of the International Society for Antiviral Research in 2004. He is currently coordinating the ANRS "HBV cure" Task Force in France and the "IP-cure-B" project within the EU H2020 work program. He co-founded the International Coalition to Eliminate HBV (ICE-HBV: http//:www.ice-hbv.org). He has published more than 570 articles (Web of Science H index 88) and was recognized by Clarivate as a highly cited researcher in 2021.

ANTONÍN HOLÝ MEMORIAL AWARDEE

Vincenzo Summa, PhD

Tracking the Journey Towards the Discovery of Raltegravir and Grazoprevir: Two Intriguing Tales on Antiviral Drug Discovery

Prof. Vincenzo Summa, PhD obtained the Master Degree in Chemistry at the University of Rome "La Sapienza" in Italy, and the PhD in organic chemistry at the University of Wuppertal in Germany. In 1996, he joined IRBM - Merck Research Laboratory in Italy, as a Research Chemist and subsequently took on positions of increasing responsibility including serving as a Director in the Medicinal Chemistry department.

Vincenzo led the chemistry team that discovered Raltegravir (Isentress™) the first in class HIV integrase inhibitor approved for the treatment of HIV / AIDS patients and Grazoprevir a pangenotype HCV protease inhibitor approved by FDA in combination with Elbasvir (Zepatier™) with "Breakthrough Therapy Designation" for HCV interferon free therapy. Isentress™ awarded the Prize Galien USA and EU for the best pharmaceutical agent in 2008.

Vincenzo was awarded as Heroes of Chemistry from American Chemical Society in 2013 for the discovery of Isentress™ and in 2017 for the discovery of Grazoprevir. He was also heavily involved in a number of other antiviral drug discovery projects aimed at the treatment of HIV infection or the cure of HCV.

In 2009, he became a cofounder of IRBM Science Park the spinoff of the Merck Research Lab in Italy where he was appointed Vice President of Drug Discovery. He continued to pursue the research activity in antivirals especially HBV and Zika and neglected disease.

Since 2019, he is full professor of Medicinal Chemistry at the **University of Naples Federico II** in the department of Pharmacy. The main areas of his research are still antivirals, rare and tropical diseases. Vincenzo is author of many reviewed published papers, and inventor on fifty-five patents, and has presented extensively at international conferences.

WILLIAM PRUSOFF MEMORIAL AWARDEE

Timothy Sheahan, PhD

Preparing for Tomorrow's Pandemics, Today through the Development of Broad-spectrum Antivirals

Dr. Timothy Sheahan is an NIH funded virologist working at the host pathogen interface to develop new methods of viral control. After receiving his bachelor's degree in Microbiology from the University of New Hampshire in 1999, he moved to Boston to try to make a career in punk rock music but soon realized that he enjoyed pipetting more than playing guitar. In 2003, he began his graduate training at UNC Chapel Hill with Dr. Ralph Baric focusing coronavirus (CoV) spillover and the design broadly acting vaccines and therapies. After postdoctoral studies on hepatitis C virus (HCV) in the laboratory of 2020 Nobel Laureate Dr. Charles M. Rice at the Rockefeller University, he became an Investigator at GlaxoSmithKline working to develop host targeting antivirals to treat acute respiratory infections. Tim became an Assistant Professor in the Department of Epidemiology in the **UNC Gillings School of Global Public Health** in 2015. Prior to the COVID-19 pandemic, Sheahan and colleagues generated preclinical proof-of-principle data that remdesivir and molnupiravir were broadly active against the CoV family suggesting these antivirals could be employed to treat future emerging CoV. This work helped accelerate the clinical testing of these antivirals in early 2020. Sheahan is currently working to develop broad-spectrum inhibitors of emerging CoV and is also developing mouse models of chronic hepacivirus infection within which to study the effect of antiviral therapy on the development of liver disease and cancer. Sheahan has been active in communicating the importance of antivirals during the pandemic in print media and on television and was even the subject of a feature in GQ Magazine. Three new human CoV have emerged in the past 20 years. Thus, the development of broadly acting therapies for CoV will remain of focus of Dr. Sheahan's research to be better prepared for tomorrow's pandemics, today.

WOMEN IN SCIENCE AWARDEE

Joanne Lemieux, PhD

Structural Studies Facilitate Antiviral Drug Development Targeting the SARS-CoV-2 Main Protease and Variants of Concern

Dr. M. Joanne Lemieux is a Professor in the Department of Biochemistry, member of the Li Ka Shing Institute of Virology, and Director of Membrane Protein Disease Research Group at the **University of Alberta** in Canada. She obtained her PhD with Dr. Da Neng Wang at New York University, where she conducted membrane protein crystallography; and conducted her PDF on protease structure and function with Dr. Michael James at the University of Alberta. As a structural biologist, she has made important contributions to understanding protein structure and function. With a diverse research portfolio, her main interests are in the study of proteases in disease states, that includes membrane embedded and viral proteases. She is currently nominated principal applicant of two CIHR grants to develop viral protease inhibitors to treat COVID19: one based on a repurposed feline drug, and a second on developing an oral formulation of a protease inhibitor. Her team is investigating antiviral drugs to develop next-generation oral formulations of SARS-CoV-2 direct acting antivirals and examining the influence of mutations found in variant of SARS-CoV-2 on antiviral function. Dr. Lemieux and is a former CIHR New Investigator and Canada Research Chair, and is the Executive Scientific Director for a submitted CBRF application for a PRAIRIE Hub for Pandemic Preparedness.

DIVERSITY AWARDEE

Blanton Tolbert, PhD

How a Love of RNA Biophysics Led to the Discovery of a Novel Antiviral Against Enteroviruses

Blanton S. Tolbert is the Rudolph and Susan Rense Professor of Chemistry at **Case Western Reserve University (CWRU)**. He is also a member of the Center for RNA Science and Therapeutics. Dr. Tolbert earned his BS degree in Chemistry from the University of SC in 1999 and his PhD in Biophysics and Structural Biology from the University of Rochester in 2007. Dr. Tolbert was a Howard Hughes Medical Institute Postdoctoral Fellow at the University of MD Baltimore County from 2007-2009, where he developed advanced NMR methods to study RNA structures that contribute to packaging of retroviral genomes. He started his independent career in 2009 as an Assistant Professor of Chemistry at Miami University in Ohio. He was promoted to Associate Professor with tenure in 2015 at Case Western Reserve University, and Professor of Chemistry in 2019. From July 2021 to October 2022, Dr. Tolbert served as the Inaugural Vice Dean of Diversity, Equity and Inclusive Excellence at the CWRU School of Medicine and the Associate Director of DEI at Case CCC. He is the acting Chairperson of the NIH Office of AIDS Research Advisory Council. On November 1, 2022, Dr. Tolbert was appointed the inaugural Vice President of Science Leadership and Culture at the Howard Hughes Medical Institute. In this capacity, he provides visionary and strategic leadership of the Center for the Advancement of Science Leadership and Culture. Dr. Tolbert supervises a diverse research group that studies biochemical mechanisms by which RNA and related retroviruses replicate within the cellular environment. Specifically, his group uses structural biophysics to characterize protein-RNA complexes that regulate viral gene expression and then leverages the knowledge gained to identify novel targets for therapeutic intervention. Dr. Tolbert is the recipient of several awards and invited lectureships.

CLOAKROOM

The cloakroom will be managed by volunteers of the UNICEF (United Nations International Children's Emergency Fund – an organization that provides humanitarian and developmental assistance to children and mother in developing countries).

Participants are encouraged to donate to UNICEF when depositing their belongings at the cloakroom. An additional donation will be made by ISAR.

unicef

The 2023 Chu Family Foundation Scholarship Awardees

ISAR and The Chu Family Foundation (TCFF) Committee are pleased to announce the winners of the 2023 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.

2023 TCFF AWARDEES

Rebekah Dickmander

CHAPEL HILL, NC, UNITED STATES

Rebekah Dickmander is a graduate student finishing up her fifth year at the University of North Carolina at Chapel Hill in the lab of Dr. Nat Moorman. She is very excited to have the opportunity to travel to UMBC to study under Dr. Seley-Radtke and expand her studies of antiviral drug development to include synthesizing "fleximers." Dr. Seley-Radtke's student, Joy Thames, will spend three weeks training Bekah on various synthetic chemistry reactions to make an adenosine analog. Following this training, Joy will return with Bekah to UNC Chapel Hill where she will teach Joy how to test the synthesized compounds in biological assays for antiviral activity and cellular toxicity. Bekah and Joy are both very grateful and excited for the opportunity afforded to them by The Chu Family Foundation Scholarship to allow them to expand their studies to include different processes in the drug development pipeline.

Selina Pasquero

TURIN, ITALY

Selina Pasquero is a post-doctoral researcher in the Department of Public Health and Pediatric Sciences at the University of Turin. After a Master degree in Medical Biotechnology in 2017, she won a 4-year PhD scholarship in Molecular Medicine that she spent in the Laboratory of Pathogenesis of Viral Infections with a project addressed to unveil the mechanisms HCMV employ to foster his replication in human cells. In the last year, during her first postdoc period, she has been mainly involved in a project aimed to investigate the antiviral activity properties of the protein arginine deiminases inhibitors against two human beta-coronaviruses, HCoV-OC43 and SARS-CoV-2. In order to learn how to work in biosafety level 3 laboratory, where highly pathogenic viruses such as SARS-CoV-2 are manipulated, she plan to use the TCFF award to visit the *Laboratory of Molecular Virology*, at the University of Milano, headed by Prof. Serena Delbue. They have extensive experience in isolating and manipulating SAR-CoV-2, with which they have worked since 2020 at the start of the pandemic.

Joy Thames

BALTIMORE, MD, UNITED STATES

Joy Elizabeth Thames completed her undergraduate studies in Chemistry at Millersville University of Pennsylvania (United States, Pennsylvania) in 2018. During her four years at Millersville, she was awarded the Board of Governor's Science and Technology Full Tuition Scholarship, as well as the Sandra A. Yeager, Ph.D. Chemistry Scholarship, and various travel awards. Upon completion of research in the chemistry department, she completed an undergraduate thesis and graduated with departmental honors.

After receiving her bachelor's degree, she became a Ph.D. student in the Seley-Radtke lab at University of Maryland, Baltimore County (United States, Maryland). Her research focuses on the synthesis of modified flexible acyclic nucleosides known as "fleximers.", which feature a carbon-carbon single bond in between the imidazole and pyrimidine moieties of the nucleobase. She uses modern organic chemistry techniques to synthesize flexible analogues of Acyclovir, Tenofovir, and Cidofovir in hopes of developing a broad-spectrum antiviral analogue. Her research also focuses on the optimization of previously synthesized fleximer analogues as well as the synthesis of their phosphate analogues to be utilized in mechanism of action studies. Most recently she was selected as one of the NIH sponsored Chemistry-Biology Interface Fellows at UMBC.

The funding provided by the Chu Family Foundation Scholarship will allow Joy to participate in a true cross-training experience in collaboration with Dr. Nathaniel Moorman and his student, Bekah Dickmander at UNC Chapel Hill. Joy's cross-training experience will allow her to take the compounds she has synthesized and learn how to test them in antiviral assays over a three-week period at UNC Chapel Hill. The goal of this unique dual-lab cross-training experience will allow Joy to better understand the drug design pipeline and further develop her skills in medicinal chemistry.

Special Thank You to Our Local Hosts

Antoine Alam
Evotec

David Durantel
Center for Infectiology Research of Lyon

Massimo Levrero
INSERM

Fabien Zoulim
Lyon University, INSERM Unit 1052

Thank You

Nihal Altan-Bonnet

Enteric Viruses Replicate in Salivary Glands and Infect Through Saliva

Dr. Nihal Altan-Bonnet heads the Laboratory of Host-Pathogen Dynamics at the **National Institutes of Health** in the USA. Her lab is comprised of a multidisciplinary team of cell biologists, virologists, environmental engineers, and computational biologists who apply their talents to understand how viruses transmit themselves effectively among hosts and establish successful infections.

Dr. Altan-Bonnet and her team have made groundbreaking discoveries that include: discovering that PI4P lipids panvirally critical for infection by RNA viruses *(Hsu et al., Cell 2010)*, discovering a novel highly virulent viral infectious unit where viruses are transmitted en bloc inside extracellular vesicles (Chen et al., Cell 2015; Santiana et al., Cell Host and Microbe 2018), discovering SARS-CoV2 and other coronaviruses use the unusual lysosomal exocytotic pathway to exit cells, disrupt antigen presentation and evade immune defenses (Ghosh et al., Cell 2020), and discovering that enteric viruses such as norovirus, rotavirus and astrovirus also replicate in salivary glands and transmit through saliva *(Ghosh et al., Nature 2022)*.

Dr. Altan-Bonnet received her PhD from The Rockefeller University. She has received the Presidential Early Career Award in Science and Engineering (PECASE), Kavli and Scialog Fellowships and been elected as Fellow to the American Academy of Microbiology. She is an Associate editor at Science Advances, Molecular Biology of the Cell and mBio journals.

Marcella Bassetto

Computer-aided Approaches Towards the Development of Small-Molecule Antivirals for Norovirus Infections

Dr. Marcella Bassetto is a medicinal organic chemist working in the drug discovery research field. She holds a MSc degree in Pharmaceutical Chemistry from Padova University, and a PhD in Medicinal Chemistry from Cardiff University, which she completed in 2013, with a thesis on the computer-aided identification and synthesis of novel antiviral agents to inhibit HCV replication. With her post-graduate and early post-doctoral work, she has applied computational methods to design, and then synthesise, novel chemical entities with pharmaceutical activities as antiviral and anticancer agents. As a research associate, she worked in the group of late Professor Chris McGuigan, the inventor of the phosphoramidate pro-drug technology for antiviral and anticancer nucleosides. After briefly joining a pharmaceutical company in Rome, where she contributed to the development of novel therapeutic agents for a range of human conditions, she returned to Cardiff University as a research associate, and in 2016 she was awarded a Sêr Cymru II individual research fellowship, to optimise new small organic molecules as potential antiviral agents for Chikungunya and Zika viruses. In September 2019, she was awarded a Lectureship in Chemistry at **Swansea University Department of Chemistry**, becoming Senior Lecturer in February 2022. Starting in February 2023, she has been appointed Senior Lecturer at Cardiff School of Pharmacy and Pharmaceutical Sciences, while maintaining an honorary position at Swansea University. Her current drug discovery research focusses on the computer-aided design and synthesis of new biologically active small molecules to treat a range of human conditions, with a major focus on viral diseases (norovirus, CHIKV, ZIKV, enteroviruses, Ebola virus, coronaviruses, arenaviruses) and inherited blinding conditions.

Ben Berkhout

HIV Keeps on Surprising Us: a CRISPR-Cas Cure Adventure and a Drug-resistance Story

Ben Berkhout (BB) studied molecular biology at the Leiden University (1976-1981), and obtained his PhD in 1986 at the same university on a research project concerning the regulation of gene expression in RNA bacteriophages, in particular translational control by means of RNA structure. He performed postdoctoral research at the Dana Farber Cancer Institute of the Harvard Medical School in the field of molecular immunology (1986-1989) and initiated HIV-1 research at the National Institutes of Health, Bethesda (Department of Molecular Microbiology, 1989-1991). Since 2016, he is also visiting professor at the University of Cagliari (Sardinia, Italy).

BB initiated a molecular virology research line in 1991 upon his return to the Netherlands and he has been at the **University of Amsterdam** and the Amsterdam University Medical Center since then. He became Head of the Laboratory of Experimental Virology and was appointed as Professor of Human Retrovirology in 2002. BB is editor-in-chief of Virus Research, editor for several journals (Retrovirology, Journal of Biomedical Science, Journal of Biological Chemistry) and editorial board member for many more. He successfully supervised 49 PhD students and was a member of 146 PhD thesis committees.

BB published over 620 peer-reviewed manuscripts on diverse topics concerning HIV replication (mechanism of transcription, reverse transcription, RNA-regulated functions), virus evolution (mechanism drug-resistance, nucleotide composition, research tool), virus discovery (human coronavirus NL63), HIV vaccine design (improved Env protein immunogen design, conditional live-attenuated HIV designs) and new antiviral therapeutic strategies (RNA interference, CRISPR-Cas). His work received more than 27,000 citations and he has an H-index of 77. BB received the Retrovirology Prize in 2008 for his pioneering research on the structure and function of the HIV-1 RNA genome.

Jinhong Chang

Development of an Orally Available Antiviral Drug for Yellow Fever

Jinhong Chang, MD, PhD, is a Professor and Principal Investigator, Laboratory of Molecular Virology and Antiviral Research, at **Baruch S. Blumberg Institute**, Hepatitis B Foundation, Doylestown, Pennsylvania, USA.

Dr. Chang received her medical education and clinical training in Infectious Diseases as well as PhD in Virology at Peking University Health Science Center, Beijing, China. She received her postdoc training in Molecular Virology at Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.

Dr. Chang has more than 20 years of research experience in the areas of molecular virology, innate immunity, and antiviral drug discovery, and has more than 100 publications in peer-reviewed journals and 6 patents. She established her independent translational research group at Drexel University, Philadelphia, Pennsylvania, in 2007 and joined Baruch S. Blumberg Institute in 2015. Her group has been focusing on the development of antiviral and innate immune modulating agents for treatment of viral infections that cause hepatitis and hemorrhagic fever.

Dr. Chang has been a member of ISAR since 2008 and currently serves as the Secretary of ISAR (2020-2024).

Maura Dandri

Challenges in Anti-Hepatitis D Virus Research: Insights From Preclinical and Clinical Studies

Maura Dandri is full Professor (W3) at the **University Medical Center Hamburg-Eppendorf**, in Germany, where she leads the Research Group Viral Hepatitis. She received her B.S. in Natural Science and PhD in Microbiology and Immunology at the University of Trieste, Italy. Her training included a Postdoctoral Fellowship at the Albert Einstein College of Medicine, New York, where she worked for four years on the woodchuck model of Hepatitis B Virus (HBV) infection within the group of Prof. C.E. Rogler. Together with Prof. Joerg Petersen she also established the first human liver chimeric mouse model of HBV infection. Awarded by a long-term EMBO fellowship, she returned to Europe to perform research at the Heinrich-Pette Institute of Experimental Virology in Hamburg, Germany.

Since 2009 she is Principal Investigator and head of the Research Group Viral Hepatitis within the Department of Internal Medicine at the University Medical Center Hamburg-Eppendorf, in Hamburg, and was awarded in 2013 by the German Research Foundation with a Heisenberg Professorship. Maura Dandri is since 2019 member of the Executive Board of the German Center for Infection Research (DZIF) and since 2021 of the Governing Board of the International Coalition to Eliminate HBV (ICE-HBV).

She has performed pioneering work by developing humanized mouse models for the study of human hepatitis viruses (HBV, HDV, HCV and HEV). Her translational research interest mainly focuses on investigating HBV cccDNA metabolism in vivo, virus-host interplay and the potential of novel therapeutic strategies, in particular against HBV and HDV. Her lab is also experienced in monitoring viral and host parameters in liver biopsy samples.

Robert Davey

Drug Repurposing at High Biocontainment: Lessons Learnt From Screening Against Ebola and SARS-CoV-2 Viruses

Dr. Robert Davey's interests are in developing small molecule therapies and understanding infection mechanism of emergent viruses such as filoviruses, arenaviruses and henipaviruses. He received his PhD in Microbiology from the University of Adelaide, Australia, in 1993. After a postdoc at Harvard Medical School he was recruited as an Assistant Professor at the University of Texas Medical Branch (UTMB) in Texas, USA. While at UTMB, in 2006, he started work with Ebola virus in the newly constructed Shope lab, the first BSL4 laboratory built at a university in the USA. He then moved to Texas Biomedical Research Institute in San Antonio in 2011 where he established a program for drug development against hemorrhagic fever viruses. In 2018 he moved to **Boston University** to the newly opened **National Emerging Infectious Diseases Laboratory (NEIDL)** where he continues to work on virus host factor identification and antiviral drug development for BSL4 pathogens. He has made significant contributions in understanding the entry mechanism of filoviruses into host cells and using the virus as a probe to better understand general cell biological processes that include macropinocytosis and autophagy. He has also worked with multiple groups to identify and characterize new small molecule scaffolds with antiviral properties, using these as potential therapies and as probes to better study infection mechanism.

PLENARY SPEAKER
Esteban Domingo

The Quasispecies Challenge: In Search of Antiviral Synergisms with Lethal Mutagens

Esteban Domingo is "ad honorem" Professor of Research at the **Spanish Research Council (CSIC)** at Centro de Biología Molecular "Severo Ochoa" (CBMSO), Madrid, Spain.He received a BSc in Chemistry (1965) and a PhD in Biochemistry (1969) from the University of Barcelona. He did postdoctoral work at the University of California (UC), Irvine, working on in vitro DNA transcription, with Dr. Robert C. Warner (1969-1973), and University of Zürich, working on genetics of bacteriophage Qβ with Dr. Charles Weissmann (1974-1977). This work permitted the first calculation of a mutation rate for an RNA virus, and the first experimental evidence of quasispecies dynamics. He joined CBMSO as staff Scientist in 1977, and was promoted to Professor of Research in 1989. With his group in Madrid they documented high mutability and quasispecies dynamics of several viruses, including the important animal pathogen foot-and-mouth disease virus (FMDV). In collaboration with John Holland (UC San Diego) they established several biological implications of quasispecies dynamics, and opened the way to lethal mutagenesis as an antiviral strategy. In recent years his research is centered on viral fitness and lethal mutagenesis, using FMDV, hepatitis C virus, and SARS-CoV-2 as model systems. He has published 450 research papers and several books and book chapters, with an h index of 82. He has received several awards including the degree of Doctor honoris causa from the Universities of Liège (Belgium) in 1999, and Bern (Switzerland) in 2004. He is a member of EMBO, the European Academy, the US National Academy of Sciences, and Vice-President of the Royal Academy of Sciences of Spain.

Rebecca Ellis Dutch

Human Metapneumovirus: New Insights, New Mechanisms, New Targets

Rebecca Ellis Dutch, PhD, is a professor in the **University of Kentucky College of Medicine** Department of Molecular and Cellular Biochemistry and the Vice Dean for Research, leading the college's research initiatives. She joined the faculty of the University of Kentucky in 2000. Her research, which has resulted in continuous National Institutes of Health (NIH) funding since 2001 and numerous other grants, manuscripts, and presentations, focuses on emerging paramyxoviruses, with a particular emphasis on viral entry, assembly, and spread. Dr. Dutch has received many recognitions and honors related to her research, including the 2015-2016 University of KentuckyUniversity Research Professor award, election as president of the American Society for Virology for 2016-2017, and selection as a Fellow for the American Academy of Microbiology and as the winner of the University of Kentucky SEC Academic Achievement Award in 2022. Dr. Dutch is very active in scientific service, including roles is an editor for Journal of Virology, Plos Pathogens, and mSphere.She has been a member of numerous grant review panels, including serving as a standing member of the NIH VIRB study section, and she is currently a standing member and chair of the NIH MID study section. Dr. Dutch is also a dedicated educator who has served as the primary mentor for 20 PhD students, four MD/PhD students, four postdoctoral scholars and 33 undergraduate researchers.

PLENARY SPEAKER
Laurent Fraisse

Addressing Viral Infections in Neglected Patient Populations: The Drugs for Neglected Diseases initiative's Efforts in HIV, HCV, COVID-19 and Pandemic Preparedness, and Dengue

Laurent Fraisse joined the **Drugs for Neglected Diseases initative (DNDi)** as Research & Development (R&D) Director and Executive Team member in October 2019. In this capacity he drives DNDi's science strategy and oversees all research and clinical activities worldwide. Laurent is an experienced biotechnology and pharmaceutical executive, and a leading expert in infectious diseases, having led R&D for large pharmaceutical firms such as Sanofi and Evotec, and served as chair of the European Federation of Pharmaceutical Industries and Associations (EFPIA) Strategic Governing Group dedicated to infectious diseases. Laurent joined Sanofi in 1999 after a ten-year tenure as a science leader in Elf Atochem. During 20 years at Sanofi, he rose to serve as Vice President for Infectious Disease R&D, addressing medical needs in bacterial, parasitic, and viral related infections. He then joined Evotec as Executive Vice President for infectious diseases with the mission to accelerate the infectious disease research pipeline development and initiate new open innovation R&D initiatives. Laurent graduated from the Ecole Nationale Supérieure d'Agronomie in Rennes, France, with a specialty in Biochemistry and was awarded a PhD in Biotechnology by the Institut National Polytechnique in Toulouse in 1993.

Franck Gallardo

Combining Autofluorescent Anchortm Tagged Viruses with High Content Imaging for the Discovery of New Broad-Spectrum Herpes Virus Inhibitors

Franck Gallardo has a PhD in Biochemistry from the Medicine Faculty of the University of Montreal, Canada (2005-2010), and receive postdoctoral training under support from ARC Foundation for Cancer Research (France) and the Human frontiers science organization working on the generation of DNA visualization tools for DSB repair imaging at the University de Toulouse, France (2010-2014). Since 2014, he has been managing **NeoVirTech** SAS' development of autofluorescent viruses for the discovery of new antiviral molecules and the measurement of disinfection procedures. Dr. Gallardo has successfully closed multiyear programs from BPI France, TTOs and private companies, from hit discovery to validation in infectious animal facilities. He is currently managing a grant from the French Defense Innovation Agency of the Army Ministry (2019-2023), developing medical counter-measures in case of poxvirus outbreak. Frank is a member of ISAR and WSV. He has 8 patents and 30 publications, including 5 journal covers.

Allison Groseth

Understanding the Arenavirus-host Cell Interface as a Guide to the Development of Novel Antiviral Approaches

Allison Groseth is a Laboratory Head at the **Friedrich-Loeffler-Institut** in Germany. She performed both her undergraduate and graduate training in Canada with a BSc in Biochemistry from the University of Victoria and a PhD in Medical Microbiology from the University of Manitoba. Her PhD work was performed with the Special Pathogens Program of the Public Health Agency of Canada and focused on studying the viral determinants underlying differences in virulence between filovirus species. Her postdoctoral work at the Philipps Universität-Marburg in Germany then focused on identifying host responses associated with arenavirus pathogenesis, particularly those regulating cell death and cytokine expression. Further work as a Staff Scientist in the Laboratory of Virology of the National Institutes of Health at Rocky Mountain Laboratories in Hamilton, Montana, focused on similar questions in the context of the emergence of highly-pathogenic orthobunyaviruses, while also looking to identify common host factors associated with infection of many unrelated hemorrhagic fever-causing pathogens. During this time, she was deployed with WHO to Monrovia, Liberia as part of the response to the 2013-2016 West African Ebola outbreak. In 2015 she was recruited to the Friedrich-Loeffler-Institut on the Isle of Riems, first as a Junior Group Leader, and since 2020 as a Laboratory Head in the Institute of Molecular Virology and Cell Biology. Her research group continues to focus on understanding the virus-host interface and particularly on developing a detailed mechanistic view of differences in host cell response regulation during infection with highly pathogenic and apathogenic arenaviruses in order to identify critical players in these processes that can serve as antiviral targets. In particular, recent areas of interest include studying the regulation of cell death and cytokine responses, and the role of kinase signaling in these processes, as well as dissecting mechanisms associated with induction (and evasion) of dsRNA-detection.

Zlatko Janeba

Fine-tuning Prodrugs of Acyclic Nucleoside Phosphonates

Dr. Zlatko Janeba of the **Institute of Organic Chemistry and Biochemistry (IOCB)** in Prague, Czech Republic, earned his PhD in chemistry in 2001 from the Institute of Chemical Technology Prague & IOCB (Prof. Antonín Holý supervision). He underwent postdoctoral trainings with Prof. Morris J. Robins (Brigham Young University, Utah) and with Prof. Paul F. Torrance (Northern Arizona University). He spent 3 years as a senior scientist at Moravek Biochemicals, Inc in California. Since 2016, he is the head of the Senior Research Group at IOCB – Medicinal Chemistry of Nucleotide Analogues. His main interest is organic & medicinal chemistry, primarily design and synthesis of modified nucleosides & nucleotides, as well as of other heterocyclic compounds, with a wide range of biological properties (e.g., antiviral, anticancer, anti-parasitic, anti-inflammatory). As for antivirals, he has been working in the field of NNRTIs, and acyclic nucleoside phosphonates and their prodrugs. Zlatko has been a member of ISAR since 2004 and currently is a member of the ISAR Board of Directors. He has been also serving the society as a reviewer of abstracts for ICAR (since 2016), and as a member of the Poster Award committee and of Women in Science committee (both since 2016). He is a member of the International Society of Nucleosides, Nucleotides and Nucleic Acids (since 2012) and of the International Society of Heterocyclic Chemistry (since 2015). He serves as an associate editor of Antiviral Chemistry & Chemotherapy of Sage journals (since 2016). He has published 112 articles with over 1,400 citations (WoS, September 2022).

Thomas Kledal

Fast and Efficient Elimination of Latent and Lytic CMV Infection

Dr. Thomas Kledal is CEO and co-founder of **Synklino A/S**, a Danish biotech company aiming to change the antiviral market by developing transformative therapies that eliminates chronic virus infections risks and enables functional cures. Dr. Kledal is an entrepreneur focused on helping transplant patients "live their life again." In his CEO role in Synklino, he strives to provide rapid relief as well as to improve long term survival for transplant recipients by seeking fast and safe eradication of cytomegalovirus (CMV) infections. The drug candidate SYN002 efficiently eliminates both lytic as well as latently infected cells in culture, and thereby potently inhibits viral replication and eradicates the virus. Synklino is currently moving the lead anti CMV drug candidate into clinical development

Previously, Dr. Kledal had established and headed the Life Science & Bioengineering Innovation Network at the Technical University of Denmark (DTU). This network is a cross-departmental innovation mechanism augmenting DTU's innovation capabilities in collaboration with the life science industries. Based at the departments of Chemistry, Biotechnology and Biomedicine, Micro- and Nanotechnology and Photonics Engineering. Before, Dr. Kledal had been Head of Section of the National Veterinary Institute at DTU (DTU-VE) where he led the Section for Virology and established a high impact research organization.

Dr. Kledal has co-authored more than 30 research papers published in journals such as Science (1997) PNAS (2015), Nature Communications (2017) and the Journal of Heart and Lung Transplant (2021).

Eric Lansdon

Structural Mechanism of Drug Resistance to L-nucleosides Conferred by the HIV-1 Reverse Transcriptase M184V Mutation

Eric Lansdon, PhD is a Senior Director at **Gilead Sciences** and the group lead for Structural Biology overseeing protein X-ray crystallography and cryo-EM. A significant portion of Eric's professional career has focused on blocking HIV Reverse Transcriptase activity with NRTIs, NNRTIs and RNase H inhibitors. During his tenure at Gilead, Eric has played an integral role in the discovery and structural biology characterization of selonsertib (ASK1 inhibitor), entospletinib (Syk inhibitor), lanraplenib (Syk inhibitor), Ainovirin (HIV-1 NNRTI), and rovafovir etalafenamide (HIV-1 NRTI). Eric earned his Bachelor's degree in Chemistry from University of California Santa Barbara and PhD in Biochemistry from University of California Davis. In addition, Eric began his career at Gilead as a Postdoctoral Scientist studying resistance mutations in HBV polymerase associated with adefovir treatment and mechanisms of inhibiting HIV-1 RNase H.

You Li

Anti-hepatovirus Activity of TENT4A/B Inhibitors

Dr. You Li is an Assistant Professor in the Department of Medicine-Infectious Diseases Division at **UNC-Chapel Hill**. He received his B.S in Biology from Tsinghua University, Beijing, China in 2005 and completed his PhD degree from Rutgers University in 2011 studying regulation of mRNA degradation. He performed his postdoc training at University of North Carolina-Chapel Hill in the field of molecular virology with Dr. Stanley Lemon, where he made significant discoveries on how host microRNAs protect hepatitis C virus (HCV) RNA from exonucleolytic degradation and promote viral replication. Dr. Li's longstanding research interest has been the interactions between RNA viruses and the host RNA metabolisms and host RNA-binding proteins, and how these interactions shape virus infection. His current project focuses on host factors of hepatitis A virus (HAV) infection. His work suggests HAV replication is dependent on the cellular TRAMP-like complex ZCCHC14-TENT4. Small molecule inhibitors of TENT4 dramatically suppress HAV replication in vitro and in mouse models of hepatitis A. Dr. Li is investigating the mechanisms underlying the essential role of TENT4 proteins in HAV life cycle and the potential application of TENT4 inhibitors as therapeutics for treatmentof severe hepatitis A infection.

Jocelyne Piret

Perspectives for the Management of CMV Infections in the New Era of Antiviral Agents

Jocelyne Piret obtained her PhD, in biological sciences, at the Catholic University of Louvain (Brussels, Belgium) in 1993. She performed two 3-year postdoctoral fellowships at the same university and at **Laval University** (Quebec City, Canada). She was appointed a project leader at the Research Center in Infectious Diseases of Laval University in 1999. Her main areas of research include the pathogenesis, prevention and treatment of herpesvirus infections. She participated in the development of microbicides for the prevention of sexually transmitted infections and of topical formulations containing antiviral agents for the treatment of cutaneous herpetic lesions. She also has a particular interest in the study of the mechanisms involved in the resistance of herpesviruses to antiviral drugs. Part of her work focusses on the study of the innate immune response during infection of the brain with herpes simplex virus and Zika virus in mouse models. In this context, she participated in the evaluation of immunomodulatory agents that could be combined with antiviral drugs to improve the outcome of herpes simplex virus encephalitis. Recently, she was involved in the study of the concept of viral interference between respiratory viruses. She has authored/co-authored 75 peer-reviewed papers and book chapters.

Xavier Saelens

Single-domain Antibodies to Control Respiratory Viruses

Xavier Saelens obtained his PhD degree from the University of Ghent (Ghent, Belgium) in 1990 in the laboratory of Walter Fiers. After postdoctoral training in the influenza research group of Willy Min Jou, and in the Molecular Signaling and Cell Death group of Peter Vandenabeele, both at Ghent University, he became an assistant professor in Molecular Virology in 2008. Currently, he is a full professor in the Department of Biochemistry and Microbiology at **Ghent University** and a principal investigator at the **VIB-UGent Center for Medical Biotechnology**.

The research team of Xavier Saelens applies modern biotechnology methods to develop new vaccines and antibody-based antivirals against human respiratory viruses such as influenza virus, respiratory syncytial virus, and coronaviruses. In addition, his group uses interactomics tools to gain new insights in the molecular interplay between host and viral factors.

Together with Walter Fiers his team pioneered the development of a universal influenza A vaccine candidate and elucidated its mechanism of protection. His group also proposed a new human respiratory syncytial vaccine candidate based on the small hydrophobic protein of this virus, which successfully passed a Phase I clinical study. In addition, the team develops single domain antibodies and formats thereof as new candidate biologics to control disease caused by respiratory viruses.

Vincent Tagliabracci

The Mechanism of RNA Capping by SARS-CoV-2

Vincent Tagliabracci received his PhD in 2010 from Indiana University School of Medicine working with Peter Roach. Shortly thereafter, he joined the laboratory of Dr. Jack Dixon at the University of California, San Diego for postdoctoral training. During his postdoctoral research, he discovered the kinases that phosphorylate secreted proteins in humans, including Fam20C, which he showed is the physiological Golgi casein kinase, an enzyme that escaped identification for many years. In 2013, Dr. Tagliabracci received a K99/R00 Pathway to Independence award from the National Institutes of Health.

In 2015 Dr. Tagliabracci joined the department of Molecular Biology as an Assistant Professor at **UT Southwestern Medical Center i**n Dallas where he was named the Michael L. Rosenberg Scholar in Medical Research and a Cancer Prevention Research Institute of Texas (CPRIT) Scholar. His laboratory has made major contributions to our understanding of non-canonical functions for protein kinases by discovering diverse and unanticipated biochemical activities that are performed by this protein superfamily. Most recently, they have discovered that the kinase-like NiRAN domain in the nsp12 protein from SARS-CoV-2 participates in a unique reaction to form the core cap structure GpppA on the viral RNA. Collectively, their work on eukaryotic, prokaryotic, and viral kinases has exposed the catalytic versatility of the protein kinase fold and suggests that atypical kinases and pseudokinases should be analyzed for alternative transferase activities.

In 2018 Dr. Tagliabracci was named a Searle Scholar. He received the NIH Director's New Innovator Award (DP2) in 2019, the Norman Hackerman Award in Chemical Research from the Welch Foundation in 2020 and a W.M Keck Foundation Medical Research Grant in 2021. In October 2021, Dr. Tagliabracci was named an investigator with the Howard Hughes Medical Institute (HHMI).

Marnix Van Loock

A Pan-serotype Antiviral to Prevent and Treat Dengue: A Journey From Discovery to Clinical Development

Marnix Van Loock is the R&D Lead for Emerging Pathogens in **Johnson & Johnson Global Public Health**, located in Beerse, Belgium. In his current role, he leads dengue compound development and coronavirus therapeutic drug discovery. Additionally, Marnix is a member of the Research and Development Committee, as well as the Development Management Committee which together steers the direction of discovery and development for Global Public Health.

In his work with Dengue, Marnix leads the clinical development of a first-in-class antiviral small molecule for the prevention and treatment of dengue, tackling a major unmet medical need. As part of his role with Coronavirus, Marnix coordinates the antiviral discovery efforts on COVID-19 and related coronaviruses; in collaboration with Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response (ASPR) at the U.S. Department of Health & Human Services. In addition, he is the project lead of the Innovative Medicines Initiative (IMI) Coronavirus Accelerated R&D in Europe (CARE) consortium.

Marnix has held roles with increasing responsibility throughout his career. In 2004, he joined Tibotec as a Scientist in the HIV Entry Discovery team. After Tibotec was acquired by Johnson & Johnson, he became a member of the HIV Integrase team, coordinating the cell-based assay development. In the 2009-2012 timeframe, Marnix was the biology project lead for the cytomegalovirus latency project. He began leading the dengue compound development starting in 2012 and coronavirus drug discovery in January 2020. Marnix has co-authored more than 25 scientific peer-reviewed publications. He is also the winner of the International Society for Antiviral Research 2019 William Prusoff Young Investigator Award.

Sean P. J. Whelan

Inhibitors of the Spike Protein of Severe Acute Respiratory Syndrome Coronavirus 2

Sean P. J. Whelan, PhD is the Marvin A. Brennecke Distinguished Professor and Head of the Department of Molecular Microbiology at **Washington University School of Medicine in St. Louis**. Whelan pioneered genetic approaches to manipulate the genome of vesicular stomatitis virus – a prototype of the non-segmented negative-sense RNA viruses a class of viruses that includes some of the most significant human, animal and plant pathogens extant. Whelan changed our understanding of gene-expression in this group of viruses through mechanistic studies of the multifunctional viral polymerase proteins and through determination of the first structure of this class of viral polymerase. This work has aided in the development of antiviral drugs against this class of important pathogens. He also engineered this relatively harmless virus to carry envelope proteins from lethal viruses – such as the ones that cause Ebola, Lassa fever, and SARS-CoV-2 – to rapidly and safely learn how such viruses infect cells and replicate. Information gleaned from such studies may help design vaccines or therapies for deadly infectious diseases. Using these tools, Whelan identified the cellular receptors for Ebola, Lassa, Lujo and Lymphocytic choriomeningitis virus revealing that receptor molecules resident on endosomal and lysosomal membranes are required for infection. Whelan's honors and awards include being named a Burroughs Wellcome Fund Investigator in Pathogenesis of Infectious Disease and a Hoffmann-LaRoche Investigator in Molecular Virology, as well as receiving a Young Innovator award from Genzyme and a MERIT award from the National Institutes of Health (NIH). In 2013, he was elected a Fellow of the American Academy of Microbiology, and in 2020 was named the LGBTQ+ Scientist of the Year by the National Organization of Gay and Lesbian Scientists and Technical Professionals for his work on emerging infectious diseases.

Monday, March 13, 2023

12:00 PM – 1:45 PM

Special Event: Women in Science Roundtable

ROSERAIE 1-2 (3rd floor)

Chaired by
Kara Carter

This event is at capacity but 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

AUDITORIUM LUMIÈRE

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

001. **The Quasispecies Challenge: In Search of Antiviral Synergisms with Lethal Mutagens**
Esteban Domingo, Ph.D., *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain*

002. **Addressing Viral Infections In Neglected Patient Populations: The Drugs for Neglected Diseases Initiative's Efforts in HIV, HCV, COVID-19 and Pandemic Preparedness, and Dengue**
Laurent Fraisse, Ph.D., *Drugs for Neglected Diseases initiative, Geneva, Genève, Switzerland*

4:15 PM – 4:30 PM

Break

FORUM 1

4:30 PM – 5:30 PM

Gertrude Elion Memorial Award Lecture

AUDITORIUM LUMIÈRE

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

003. **Discovery science for the cure of Hepatitis B virus infection**
Fabien Zoulim, M.D., Ph.D., *INSERM, Lyon University, Hospices Civils de Lyon, Lyon, France*

5:30 PM – 6:30 PM

Opening Reception

FORUM 1

Tuesday, March 14, 2023

8:30 AM – 9:15 AM

William Prusoff Memorial Award Lecture

AUDITORIUM LUMIÈRE

Chaired by

Luis M. Schang and **Kathie Seley-Radtke**

004. **Preparing for Tomorrow's Pandemics, Today Through the Development of Broad-spectrum Antivirals**
Timothy P. Sheahan, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States*

9:15 AM – 10:15 AM

SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals I

AUDITORIUM LUMIÈRE

Chaired by
Mindy Davis and **Kathie Seley-Radtke**

005. **Development of an Orally Available Antiviral Drug for Yellow Fever**
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

006. **A Pan-serotype Antiviral in Early Clinical Development for the Prevention and Treatment of Dengue: A Journey From Discovery to Clinical Development Driven by Public-private Partnerships**
Marnix Van Loock, Ph.D., *Global Public Health R&D, Janssen Pharmaceutica NV, Beerse, Belgium*

10:15 AM – 10:30 AM

Break

FORUM 1

10:30 AM – 11:00 AM

SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals I (continued)

AUDITORIUM LUMIÈRE

Chaired by
Dahai Luo and **María Jesús Pérez Pérez**

007. **The mechanism of RNA capping by SARS-CoV-2**
Vincent Tagliabracci, Ph.D., *HHMI/UT Southwestern, Dallas, TX, United States*

11:00 AM – 12:00 PM

PechaKucha Competition

AUDITORIUM LUMIÈRE

Chaired by
Kathie Seley-Radtke

12:00 PM – 1:30 PM

Special Event: Career Development Interactive Roundtable

ROSERAIE 1-2 (3rd floor)

Chaired by
Leen Delang

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

2:00 PM – 2:45 PM

Women in Science Award Lecture

AUDITORIUM LUMIÈRE

Chaired by
Kathie Seley-Radtke

009. **Structural Studies Facilitate Antiviral Drug Development Targeting the SARS-CoV-2 Main Protease and Variants of Concern**
Joanne Lemieux, Ph.D., *University of Alberta, Edmonton, AB, Canada*

2:45 PM – 3:45 PM

Chronic, Persistent, or Latent Viruses I

AUDITORIUM LUMIÈRE

Chaired by
Jinhong Chang and **David Durantel**

010. **Fast and Efficient Elimination of Latent and Lytic CMV Infection**
Thomas N. Kledal, Ph.D., *Synklino A/S, Copenhagen, Copenhagen, Denmark*

011. **Challenges in Anti-Hepatitis D Virus Research: Insights from Preclinical a nd Clinical Studies**
Maura Dandri, Ph.D., *University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

3:45 PM – 4:00 PM

Break

FORUM 1

4:00 PM – 5:00 PM

Chronic, Persistent, or Latent Viruses I (continued)

AUDITORIUM LUMIÈRE

Chaired by
Kara Carter and **Fabien Zoulim**

012. **Structural Mechanism of Drug Resistance to L-nucleosides Conferred by the HIV-1 Reverse Transcriptase M184V Mutation**
Eric Lansdon, Ph.D., *Gilead Sciences, Foster City, California, United States*

013. **Fine-Tuning Prodrugs of Acyclic Nucleoside Phosphonates**
Zlatko Janeba, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*

5:00 PM – 7:00 PM

Poster Session 1

FORUM 1

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

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

Light food and beverages will be provided.

Wednesday, March 15, 2023

8:15 AM – 8:30 AM

Tribute to Mike Bray

8:30 AM – 10:00 AM

Influenza, RSV, and Other Respiratory Viruses

AUDITORIUM LUMIÈRE

Chaired by
Anne Moscona and **Marco Vignuzzi**

014. **Human Metapneumovirus: New Insights, New Mechanisms, New Targets**
Rebecca Ellis Dutch, Ph.D., *University of Kentucky College of Medicine, Lexington, Kentucky, United States*

015. **Inhibitors of the Spike Protein of Severe Acute Respiratory Syndrome Coronavirus 2**
Sean P.J. Whelan, Ph.D., *Department of Molecular Microbiology, School of Medicine, Washington University in St. Louis, St. Louis, MO, United States*

016. **Single-domain Antibodies to Control Respiratory Viruses**
Xavier Saelens, Ph.D., *VIB-UGent Center for Medical Biotechnology; Department of Biochemistry and Microbiology, Ghent, Belgium*

10:00 AM – 10:15 AM

Break

FORUM 1

10:15 AM – 11:30 AM

Influenza, RSV, and Other Respiratory Viruses (continued)

AUDITORIUM LUMIÈRE

Chaired by
Cybele Garcia and **Brett Hurst**

017. **A Novel SARS-CoV-2 Inhibitor Targeting the Membrane Protein With Activity in a SCID Mouse Model**
Manon Laporte, Ph.D., *KU Leuven, Leuven, Belgium*

018. **Biochemical and Structural Insights Into SARS-CoV-2 Polyprotein Processing by Mpro: Implications for Developing Novel Antiviral Strategies**
Ruchi Yadav, M.S., *Rutgers University, New Brunswick, Piscataway, NJ, United States*

019. **Bemnifosbuvir (BEM, AT-527) a Potent Inhibitor of SARS-CoV-2 Variants of Concern (VOC), and a Promising Oral Antiviral with a High Resistance Barrier for Treatment of COVID-19 and other Coronaviruses Infections**
Qi Huang, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*

020. **Combination of Antiviral Drugs Targeting SARS-CoV-2 RNA Polymerase and Exonuclease in vitro Demonstrates COVID-19 Therapeutic Potential**
Carolina Sacramento, Ph.D., *Fiocruz, Rio de Janeiro, Brazil*

021. **EDP-235, an Oral 3CL Protease Inhibitor for the Treatment of COVID-19, Suppresses Viral Replication and Spread in SARS-CoV-2-Infected Ferrets**
Michael Rhodin, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*

022. **A Hinge Glycan Regulated Spike Bending Impacts Coronavirus Infectivity**
Jing Jin, Ph.D., *Vitalant Research Institute, San Francisco, CA, United States*

023. **Development of Small Molecule Entry Inhibitors of Influenza A Viruses**
Jazmin Galvan Achi, Ph.D. Seeking, *University of Illinois Chicago, Chicago, Illinois, United States*

11:30 AM – 12:15 PM

Late-breaking Oral Presentations

AUDITORIUM LUMIÈRE

Chaired by
Robert Jordan and **Angela Corona**

024. **Intermittent Therapy with IM 250, A Helicase Primase Inhibitor, Has Persistent Effects and May Reduce the Pool of latent Reactivable Herpes Simplex Virus**
Gerald Kleymann, Ph.D., *Innovative Molecules GmbH, Munich, Bavaria, Germany*

025. **Intranasal delivery of fusion inhibitory lipopeptides blocks SARS-CoV-2-induced pathology in mice and permits establishment of long-lasting protective immunity**
Branka Horvat, M.D., Ph.D., *International Center for Infectiology Research (CIRI), Lyon, France*

026. **Ebola Virus Disease: In Vivo Protection Provided by the PAMP Restricted TLR3 Agonist Rintatolimod and Its Mechanism of Action**
Angela Corona, Ph.D., *Molecular Virology Unit Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*

027. **Drug deconstructing and re-engineering as an alternative to drug repurposing**
Consuelo B. Correa-Sierra, M.D., Ph.D., *Cornell University, Ithaca, NY, United States*

12:15 PM – 2:15 PM

Poster Session 2

FORUM 1

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

2:15 PM – 3:15 PM
EVEN numbered posters

Lunch will be provided.

Thursday, March 16, 2023

8:30 AM – 9:15 AM

Antonín Holý Memorial Award Lecture

AUDITORIUM LUMIÈRE

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

028. **Tracking the Journey Towards the Discovery of Raltegravir and Grazoprevir: Two Intriguing Tales On Antiviral Drug Discovery**
Vincenzo Summa, Ph.D., *Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

9:15 AM – 10:15 AM

Chronic, Persistent, or Latent Viruses II

AUDITORIUM LUMIÈRE

Chaired by
Antoine Alam and **Graciela Andrei**

029. **Perspectives for the Management of Cytomegalovirus Infections in the New Era of Antiviral Agents**
Jocelyne Piret, Ph.D., *CHU de Quebec-Laval University, Quebec City, Quebec, Canada*

030. **HIV Keeps on Surprising Us: a CRISPR-Cas Cure Adventure and a Drug-resistance Story**
Ben Berkhout, Ph.D., *University of Amsterdam, Amsterdam, Netherlands*

10:15 AM – 10:30 AM

Break

FORUM 1

10:30 AM – 12:00 PM

Chronic, Persistent, or Latent Viruses II (continued)

AUDITORIUM LUMIÈRE

Chaired by
Chris Meier and **Jennifer Moffat**

031. **Combining Autofluorescent ANCHORTM Tagged Viruses with high Content Imaging for the Discovery of New Broad-spectrum Herpes Virus Inhibitors**
Franck Gallardo, Ph.D., *NeoVirTech SAS, Toulouse, France*

032. **High Throughput Discovery of Small Molecular Inhibitors of Hepatitis B Virus Subviral Particle Biogenesis**
Biplav Shrestha, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

033. **Cytosine Base Editing Inactivates the Hepatitis B Virus Episomal Genomic Reservoir and Integrated DNA**
Anuj Kumar, Ph.D., *Cancer Research Center of Lyon, INSERM, Lyon, France*

034. **Optimization and Validation of a Rat HEV Transmission Model for Pre-clinical Evaluation of Novel Antiviral Molecules**
Xin Zhang, M.S., *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

035. **The Complex of NBD-14189 with HIV-1 Reverse Transcriptase and DNA Reveals its Molecular Mechanism of Inhibition of Reverse Transcription**
Natalie Losada, Ph.D. Seeking, *Center for Advanced Biotechnology and Medicine; Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey, United States*

036. **FXR Agonists Alone or in Combination with IFNa Inhibit HBV Replication and HDV Propagation in Functional Hepatocytes**
Romain Barnault, Ph.D., *HepVir – CIRI – Inserm, Lyon, France*

037. **On Exploring the Structure-Activity Relationship of Nucleoside Phosphonates as Hepatitis B Virus (HBV) Inhibitors**
Elisabetta Groaz, Ph.D., *Rega Institute, Medicinal Chemistry, Leuven, Belgium*

12:00 PM – 12:15 PM

ISAR Annual Business Meeting

AUDITORIUM LUMIÈRE

PRESIDENT: **Kathie Seley-Radtke**
TREASURER: **Brian Gowen**
SECRETARY: **Jinhong Chang**

2:00 PM – 2:45 PM

Diversity Speaker Award Lecture

AUDITORIUM LUMIÈRE

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

038. **How a Love of RNA Biophysics Led to the Discovery of a Novel Antiviral Against Enteroviruses**
Blanton S. Tolbert, Ph.D., *Case Western Reserve University, Cleveland, Ohio, United States*

2:45 PM – 3:45 PM

SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals II

AUDITORIUM LUMIÈRE

Chaired by
Andrea Brancale and **Jessica Spengler**

039. **Understanding the Arenavirus-Host Cell Interface as a Guide to the Development of Novel Antiviral Approaches**
Allison Groseth, Ph.D., *Friedrich-Loeffler-Institut, Greifswald – Insel Riems, Germany*

040. **Drug Repurposing at High Biocontainment: Lessons Learnt From Screening Against Ebola and SARS-CoV-2 Viruses**
Robert A. Davey, Ph.D., *National Emerging Infectious Diseases Laboratories, Boston University, Boston, MA, United States*

3:45 PM – 4:00 PM

Break

FORUM 1

4:00 PM – 6:00 PM

SARS-CoV-2, Arboviruses, Other Biothreat Viruses, and Broad Spectrum Antivirals II (continued)

AUDITORIUM LUMIÈRE

Chaired by
Zlatko Janeba and **Christina Spiropoulou**

041. **Design of SARS-CoV-2 PLpro Inhibitors for COVID-19 Antiviral Therapy Leveraging Binding Cooperativity**
Laura Cooper, Ph.D., *University of Illinois at Chicago, Chicago, IL, United States*

042. **Discovery of a Novel SARS-Cov2 Helicase Inhibitor from a 100K HTS Campaign**
Donghoon Chung, Ph.D., *University of Louisville, Louisville, KY, United States*

043. **The Viral Non-Structural Proteins as Antiviral Targets: From Screening to Hit Validation**
Jean-Claude Guillemot, Ph.D., *AIX Marseille University, Marseille, France*

044. **Antiviral Activity of Viperin-Inspired 3′-Deoxy-3′,4′-didehydro-nucleoside Phosphoramidate Prodrugs**
Samantha Kennelly, Ph.D. Seeking, *Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States*

045. **JNJ-A07 Targets the Dengue Virus NS4A-2K-NS4B Interaction with NS3 and Blocks De Novo Formation of Vesicle Packets**
Dominik Kiemel, Ph.D. Seeking, *Heidelberg University, Heidelberg, Baden-Württemberg, Germany*

046. **AT-752 Targets Multiple Sites and Activities on the Dengue Virus Replication Enzyme NS5**
Mikael Feracci, Ph.D., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*

047. **Five Cellular Enzymes in the Activation Pathway of Bemnifosbuvir, a Drug-candidate Against SARS-CoV-2 infections**
Aurélie Chazot, M.S., *AFMB UMR 7257, Marseille, France*

048. **Treatment of Yellow Fever Virus with the NS4B Inhibitor BDAA and Effects on RNA-Sensing Innate Immune Pathways in a Hamster Model**
Abbie E. Weight, B.S., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

049. **New Class of Small Molecules that Inhibits Yellow Fever Virus by Targeting the NS4B Protein**
Alina Soto, Ph.D. Seeking, *KU Leuven, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium*

050. **Molecular Architecture of the Chikungunya Virus Replication Complex for Antiviral Development**
Dahai Luo, Ph.D., *Nanyang Technological University, Singapore, Singapore*

051. **The MEK1/2 inhibitor Zapnometinib Is Safe and Well Tolerated In Humans and Has Both, Anti-SARS-CoV-2 As Well As Immunomodulatory Activity**
Oliver Planz, Ph.D., *Eberhard Karls University, Tübingen, Germany*

052. **AI-driven Approach to the Discovery of novel Mpro Inhibitors with High Pan-coronavirus Activity**
Marco Derudas, Ph.D., *Exscientia, Oxford, United Kingdom*

6:00 PM – 8:00 PM

Closing Event

FORUM 1

Friday, March 17, 2023

9:00 AM – 10:30 AM

Acute GI Viruses

AUDITORIUM LUMIÈRE

Chaired by
Brian Gowen and **Joana Rocha-Pereira**

053. **Enteric Viruses Replicate In Salivary Glands and infect Through Saliva**
Nihal Altan-Bonnet, Ph.D., *National Institutes of Health, USA, Bethesda, Maryland, United States*

054. **Computer-aided Approaches Towards the Development of Small-molecule Antivirals for Norovirus Infections**
Marcella Bassetto, Ph.D. Seeking, *Swansea University, Swansea, Wales, United Kingdom*

055. **Anti-hepatovirus Activity of TENT4A/B Inhibitors**
You Li, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

10:30 AM – 10:45 AM

Break

FORUM 1

10:45 AM – 12:00 PM

Chronic, Persistent, or Latent Viruses III

AUDITORIUM LUMIÈRE

Chaired by
Graciela Andrei and **Jennifer Moffat**

056. **Differential Dynamics and Evolution of Cytomegalovirus Infection in Transplant Recipients Grafted With Organs Derived From the Same Donor**
Fien Horsten, Ph.D. Seeking, *KU Leuven, Leuven, Belgium*

057. **Two Novel Small Chemical Compounds Blocking Herpes Simplex Virus Assembly**
Julio Cesar Villalvazo Guerrero, Ph.D., *Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover-Braunschweig Site, Hannover, Germany*

058. **Herpesvirus-mediated Protein Citrullination as A New Target for Antiviral Therapy**
Selina Pasquero, Ph.D., *Department of Public Health and Pediatric Sciences, University of Turin – Medical School, Turin, Italy*

059. **Combined gB (humoral) and IE1 (cell-mediated) Ad Vector CMV Vaccines Are More Effective Than Disabled CMV DISC Vaccine for Cross Strain Protection Against Congenital Cytomegalovirus Disease**
Alistair McGregor, Ph.D., *Texas A&M University Health Science Center, Bryan, TX, United States*

060. **Different Epigenetic Inhibitors Targeting Chromatin Remodeling Complexes Inhibit or Activate HSV-1 Replication**
Sarah Saddoris, Ph.D. Seeking, *Cornell University, Ithaca, NY, United States*

061. **POM-L-BHDU is a Highly Potent Prodrug of L-Dioxolane Bromovinyl Uridine That Prevents Varicella Zoster Virus Spread Topically In Skin Organ Culture and Orally In NuSkin Mice**
Megan Lloyd, Ph.D., *SUNY Upstate Medical University, Syracuse, NY, United States*

062. **Identification of 27-Hydroxycholesterol Synthetic Analogs as a Novel Class of Anti-Herpes Simplex Virus Antivirals**
Andrea Civra, Ph.D., *University of Turin – Department of Clinical and Biological Sciences, Orbassano, Turin, Italy*

12:00 PM – 12:30 PM

Shotgun Presentations and Closing Remarks

AUDITORIUM LUMIÈRE

Chaired by
Two Trainees TBD

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

020.* **Combination of Antiviral Drugs Targeting SARS-CoV-2 RNA Polymerase and Exonuclease in vitro Demonstrates COVID-19 Therapeutic Potential**
Carolina Sacramento, Ph.D., *Fiocruz, Rio de Janeiro, Brazil*

023.* **Development of Small Molecule Entry Inhibitors of Influenza A Viruses**
Jazmin Galvan Achi, Ph.D. Seeking, *University of Illinois Chicago, Chicago, Illinois, United States*

027.* **Drug Deconstructing and Re-engineering as an Alternative to Drug Repurposing**
Consuelo B. Correa-Sierra, M.D., Ph.D., *Cornell University, Ithaca, NY, United States*

033.* **Cytosine Base Editing Inactivates the Hepatitis B Virus Episomal Genomic Reservoir and Integrated DNA**
Anuj Kumar, Ph.D., *Cancer Research Center of Lyon, INSERM, Lyon, France*

036.* **FXR Agonists Alone or In Combination with IFNa inhibit HBV Replication and HDV Propagation In Functional Hepatocytes**
Romain Barnault, Ph.D., *HepVir – CIRI – Inserm, Lyon, France*

041.* **Design of SARS-CoV-2 PLpro Inhibitors for COVID-19 Antiviral Therapy Leveraging Binding Cooperativity**
Laura Cooper, Ph.D., *University of Illinois at Chicago, Chicago, IL, United States*

042.* **Discovery of a Novel SARS-Cov2 Helicase Inhibitor from a 100K HTS Campaign**
Donghoon Chung, Ph.D., *University of Louisville, Louisville, KY, United States*

047.* **Five Cellular Enzymes In the Activation Pathway of Bemnifosbuvir, A Drug-candidate Against SARS-CoV-2 Infections**
Aurélie Chazot, M.S., *AFMB UMR 7257, Marseille, France*

048.* **Treatment of Yellow Fever Virus with the NS4B Inhibitor BDAA and Effects on RNA-Sensing Innate Immune Pathways in a Hamster Model**
Abbie E. Weight, B.S., *Institute for Antiviral Research, Utah State University, Logan, UT, United States*

049.* **New Class of Small Molecules that Inhibits Yellow Fever Virus by Targeting the NS4B Protein**
Alina Soto, Ph.D. Seeking, *KU Leuven, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium*

056.* **Differential Dynamics and Evolution of Cytomegalovirus Infection in Transplant Recipients Grafted With Organs Derived From the Same Donor**
Fien Horsten, Ph.D. Seeking, *KU Leuven, Leuven, Belgium*

058.* **Herpesvirus-mediated Protein Citrullination As A New Target for Antiviral Therapy**
Selina Pasquero, Ph.D., *Department of Public Health and Pediatric Sciences, University of Turin – Medical School, Turin, Italy*

**Also presenting a short oral presentation*

060.* **Different Epigenetic Inhibitors Targeting Chromatin Remodeling Complexes Inhibit or Activate HSV-1 Replication**
Sarah Saddoris, Ph.D. Seeking, *Cornell University, Ithaca, NY, United States*

062.* **Identification of 27-Hydroxycholesterol Synthetic Analogs as a Novel Class of Anti-Herpes Simplex Virus Antivirals**
Andrea Civra, Ph.D., *University of Turin – Department of Clinical and Biological Sciences, Orbassano, Turin, Italy*

100. **A Gut-immune Model System Using Human Intestinal Enteroids to Identify Antivirals Targeting Enteric Viruses and the Host Immune Response**
Jana Van Dycke, Ph.D., *KU Leuven – Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

101. **High-content Antiviral Screening Assay for Enteric Viruses Using Human Intestinal Organoids**
Joana Rocha-Pereira, Ph.D., *KU Leuven, Department of Microbiology, Immunology & Transplantation, Rega Institute, Laboratory of Virology & Chemotherapy, Leiden, Belgium*

103. **Identification of Dctn6, a Druggable Host Factor Exploited by Enterovirus-71 During Its Infection Cycle**
Elijah Chen, Ph.D., *NUS, Singapore*

104. **Norovirus NS1-2 as a Target for the Computer-aided Identification of Novel Antiviral Agents**
Salvatore Ferla, Ph.D., *Swansea University Medical School, Swansea, United Kingdom*

200. **1 Prophylactic and Therapeutic Efficacy of a Novel Brain-penetrant Antiviral in 2 Lethal Mouse Models of Venezuelan and Eastern Equine Encephalitis**
Colleen Jonsson, Ph.D., *University of Tennessee Health Science Center, Memphis, TN, United States*

201. **Antiviral Effect of Atovaquone against Chikungunya and Zika Virus in Aedes aegypti Mosquitoes by Tarsal Exposure via the Mosquito Legs**
Leen Delang, Ph.D., *Rega Institute, University of Leuven, Leuven, Belgium*

202. **Antiviral Strategies for Encephalitic Viral Diseases**
Karin Peterson, Ph.D., *Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States*

203. **Dengue Virus Infection and Dissemination Are Disrupted in Aedes aegypti Mosquitoes Exposed to JNJ-A07 in the Bloodmeal**
Ana L. Rosales Rosas, M.S., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

204. **Development of Proximity-based Antivirals: Mechanisms Beyond Targeted Protein Degradation**
Priscilla Yang, Ph.D., *Stanford University School of Medicine, Stanford, California, United States*

**Also presenting a short oral presentation*

205. Discovery and Synthesis of 1,2,4-oxadiazole Derivatives as Novel Inhibitors of Zika Virus Infection
Yun Young Go, D.V.M., Ph.D., *City University of Hong Kong, Hong Kong (SAR China)*

206. Efficient Incorporation of 2′-Fluoro,2′-Bromouridine Triphosphate Inhibits Yellow Fever Virus Polymerase Selectively
Calvin Gordon, Ph.D. Seeking, *Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada*

207. Viral Kinetics during Acute Chikungunya Virus Infection and the Potential for Antiviral Treatment
Hugh Watson, Ph.D., *Evotec ID (Lyon), Lyon, Rhone, France*

208. Identification of Novel Regulatory Sites of Alphavirus Non-structural Protein 2 Activity Via Alphafold2 Informed Mutagenesis
Jack Sears, Ph.D. Seeking, *University of North Carolina – Chapel Hill, Department of Microbiology and Immunology, Chapel Hill, NC, United States*

209. Molecular Architecture of Chikungunya Virus Replication Complexes for Antiviral Drug Discovery
Yaw Bia Tan, Ph.D. Seeking, *Nanyang Technological University, Singapore*

210. Potent Primate-specific Pan-dengue Inhibitors
Chin Piaw Gwee, M.S., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*

211. Reduced Dengue Virus Replication Upon Oral Exposure of Aedes aegypti Mosquitoes to the Antiviral Nucleoside Analog 7DMA
Febrina Meutiawati, M.S., *Radboud University Medical Center, Jakarta Selatan, DKI Jakarta, Indonesia*

212. Screening and Phytochemical Characterization of Endemic Plants from Madagascar and Reunion Island for Antiviral Activity Against Zika and Dengue Virus
Chaker El Kalamouni, Ph.D., *PIMIT, Processus Infectieux en Milieu Insulaire Tropical, Université de La Réunion, INSERM U1187, CNRS 9192, IRD 249, plateforme CYROI 97490 Sainte-Clotilde, La Réunion, France*

213. The Lipid Liaisons of Secreted Dengue Virus NS1 – A Structural Insight
Subhash Vasudevan, Ph.D., *Duke-NUS Medical School, Singapore*

214. Tick-Borne Encephalitis Virus-infected Human Neuronal/Glial Cells Identify Antiviral Drugs
Muriel Coulpier, Ph.D., *INRAE, Maisons-Alfort, France*

215. Tissue-specific Expansion of Isogenic Variants in the ZIKV Quasispecies Drive Disease Pathogenesis
Kitti W. K. Chan, Ph.D., *Duke-NUS Medical School, Singapore*

216. Trehalose Monolaurate Suppresses Dengue Virus Infection Through Multiple Mechanisms
Yi-Jung Ho, Ph.D., *National Defense Medical Center, Taipei, Taiwan, Republic of China*

217. Trehalose-based Esters Possess an Antiviral Ability toward Sindbis Virus
Yen-Chen Chen, Pharm.D., *Institute of Preventive Medicine, Taipei, Taiwan, Republic of China*

218. Viral RNA Decapping Enzyme Chikungunya Virus nsP1 Activates Type I Interferon Signalling Pathway
Michelle Law, Ph.D. Seeking, *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*

219V. A Yellow Fever Virus NS4B Inhibitor Executes Multiple Mode-of-Action
Fuxuan Wang, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

220V. Trans-dominant Inhibition of DENV Replication Mediated by a NS3 Internal Cleavage Site
Milly Choy, Ph.D., *Duke-NUS Medical School, Singapore*

300. A lipopeptide Fusion-inhibitor Platform for Preventing and Treating Enveloped Viral Infection
Matteo Porotto, Ph.D., *Columbia University Vagelos College of Physicians & Surgeons, New York, NY, United States*

301. A Systematic Study of SARS-CoV-2 Main Protease Drug Resistant Mutants Against Nirmatrelvir and Ensitrelvir
Jun Wang, Ph.D., *Rutgers University: Rutgers The State University of New Jersey, Piscataway, New Jersey, United States*

302. Advanced Junín Mammarenavirus Infection and Disease Effectively Treated with the Broadly Active Ribonucleoside Analog, EIDD-2749
Jonna Westover, Ph.D., *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT, United States*

303. Analytical Methods Development for the Measurement of Physico-chemical Parameters of a New Antiviral in Pre-clinical Phase
Luigi Agrofoglio, Ph.D., *ICOA UMR CNRS 7311, Orléans, France*

304. Synthesis of Heterocyles Targeting STING Protein in Innate Immunity
Luigi Agrofoglio, Ph.D., *ICOA UMR CNRS 7311, Orléans, France*

305. Application of Virtual Screening Methods for the Identification of novel Ebola Virus (EBOV) VP24 Inhibitors
Alessia Onali, *University of Cagliari, Cagliari, Italy*

306. Approaches to Develop icDNA Clones of Encephalitic Alphaviruses to Characterize Viral Disease and Evaluate Broad-spectrum Therapeutics
Rachel Ireland, Ph.D., *DSTL, Salisbury, United Kingdom*

307. Assay Development for High-throughput Antiviral Compound Screening Against bunyavirus L Protein
Dominik Vogel, Ph.D., *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

308. Broad-spectrum Antiviral Activity of Polyoxometalates
Manuela Donalisio, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*

309. Combinations of Host- and Virus-targeting antiviral Drugs Confer Synergistic Suppression of SARS-CoV-2
Stephen Polyak, Ph.D., *University of Washington, Seattle, WA, United States*

310. Crystal Structure of the 2′-O-ribose Methyltransferase VP39 from Monkeypox Virus in Complex with Sinefungin
Jan Silhan, Ph.D., *Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic*

311. Deep Sequencing Reveals Correlates of Prophylactic Protection of BDGR-49 in Mice Intranasally Challenges with Venezuelan Equine Encephalitis Virus Trinidad Donkey
Evan Williams, Ph.D., *Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, United States*

312. Design, Synthesis, and Biological Evaluation of Expanded (Linker) Flex-Acyclovir Analogues as Potential Broad-Spectrum Antiviral Drugs
Joy E. Thames, Ph.D. Seeking, *University of Maryland, Baltimore County, Baltimore, MD, United States*

313. Development of a Cell-Based Assay for Evaluating b-coronavirus inhibitors
Rebekah J. Dickmander, *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

314. Development of a Virucidal Compound Against Influenza Viruses
Caroline Tapparel, *Université de Genève, Genève, Switzerland*

315. Development of Molecularly Defined Broad-spectrum Virucidal Drugs
Yong Zhu, Ph.D. Seeking, *Institute of Materials Science & Engineering, Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

316. Druggability Assessment of the Bunyaviral Cap-binding Domain
Janna Scherf, Ph.D. Seeking, *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

317. Efficay of tecovirimat, Brincidofovir, and Cidofovir Against a Human Monkeypox Virus Isolate from the Currently Ongoing Epidemic in Europe
Graciela Andrei, Ph.D., *KU Leuven, Leuven, Belgium*

318. Generation and Characterization of Recombinant MA-EBOV Expressing Reporter Proteins in vitro and in vivo for use in Therapeutic and Vaccine Efficacy Studies
Katherine Davies, Ph.D., *CDC, Atlanta, Georgia, United States*

319. High-Throughput Fluorescent Assay for Inhibitor Screening of Proteases from RNA Viruses
Andrea Huskova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

320. Identification and Evaluation of Novel Macrocyclic Compounds Against Hemorrhagic Fever Arenaviruses
Virginia Aida-Ficken, D.V.M., *The Centers for Disease Control & Prevention, Atlanta, GA, United States*

321. Implementation of a Preclinical Platform for the Evaluation of Antivirals During an Emergence Period: the Example of SARS-CoV-2
Jean-Sélim Driouich, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*

322. In vitro Evaluation of Double Combinations Against Coxsackievirus B3 and Poliovirus Type 1
Adelina Stoyanova, Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

323. In vitro Screening for Inhibitors of the 2′-O-ribose Methyltransferase VP39 from mpox Virus
Dominika Chalupska, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

324. Treatment via Consecutive Alternating Administration of Antivirals Against Coxsackievirus B3 Infection in Mice
Adelina Stoyanova, Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

325. In vitro Studies of the Barrier to Resistance of Sofosbuvir Against Tick-borne Encephalitis and Yellow Fever Viruses
Alekxander Binderup, M.S., *Copenhagen Hepatitis C Program (CO-HEP), University of Copenhagen and Hvidovre Hospital, Copenhagen, Denmark*

326. Inhibitors of Coronavirus and Monkeypox Virus Methyltransferases: Similarities and Differences
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic*

327. Leveraging the Power of Artificial Intelligence to Discover Drugs for Pandemic Preparedness
Fraser Cunningham, Ph.D., *Exscientia, Dundee, United Kingdom*

328. Mechanisms of Action of Repurposed Ebola Virus Antivirals
Jamie Kelly, *Centers for Disease Control and Prevention, Atlanta, GA, United States*

329. Modeling SARS-COV-2-infected Central Nervous System Using Human Primary Neuronal/glial Cells to Identify Antiviral Drugs
Noemie Berry, Ph.D., *INRAE, Maisons-Alfort, France*

330. Modulation of the Aryl Hydrocarbon Receptor Signaling Pathway Impacts on Junín Virus Replication
Miguel Ángel Peláez, Ph.D. Seeking, *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*

331. Nebulization of Fusion Inhibitory Lipopeptide, A Way to Protect Nonhuman Primates Against Respiratory Nipah Virus Infection
Olivier Reynard, Ph.D., *CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS, UMR5308, Univ Lyon, Université Claude Bernard Lyon 1, École Normale Supérieure de Lyon, Lyon, Rhone, France*

332. Non-nucleotide RNA-dependent RNA-polymerase Inhibitor as Potential Antiviral Drug
Katerina Krejcova, M.S., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic*

333. Peptoid Amphiphiles as Membrane Active Antivirals
Patrick Tate, B.S., *New York University, New York, New York, United States*

334. Plitidepsin is a Host-Directed Antiviral that Transiently Inhibits Protein Translation of Distant Viruses while Shaping a Protective Proteostatic Cellular Response
Nuria Izquierdo-Useros, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*

335. Potent and Structurally Distinct Antiviral Hits against Monkeypox Virus
Zhengqiang (ZQ) Wang, Ph.D., *Center for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, United States*

336. Pre-Clinical Evaluation and Mode of Action of Molnupiravir against SARS-CoV-2
Paul-Rémi Petit, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*

337. PROTAC Approach for Antiviral Drug Discovery
Xicheng Sun, Ph.D., *Crestone Inc., Boulder, CO, United States*

339. RNA Helicase eIF4A Inhibitors as Host-targeting Pan-antivirals
Arnold Grünweller, Ph.D., *Philipps University Marburg, Institute of Pharmaceutical Chemistry, Marburg, Germany*

340. Small Molecule Entry Inhibitors of Ebola and Marburg Filoviruses
Malaika Argade, Ph.D., *UICentre, College of Pharmacy, University of Illinois, Chicago, Chicago, IL, United States*

341. Structural Characterisation of the Monkeypox Poxin in an Unliganded Form and Bound to a Novel Cyclic Dinucleotide MD1203
Vojtech Duchoslav, Ph.D. Seeking, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*

342. Synergistic Inhibitory Effect of Remdesivir and Ribavirin against SARS-CoV-2
Carlos García-Crespo, Ph.D. Seeking, *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*

343. Synthesis of Novel Antiviral Nucleoside Phosphoramidates Targeting Viral Polymerases
Jacob Sawyer, Ph.D. Seeking, *University of Minnesota, Minneapolis, MN, United States*

344. The Human DEAD-box RNA Helicase eIF4A as a Promising Pan-antiviral Target – Mechanistic Aspects and Fragment-based Development of New eIF4A Inhibitors
Francesca Magari, Ph.D., *Philipps-Universität Marburg, Marburg (Lahn), Germany*

345. Towards Immunotherapy against Alphaviral Encephalitis
AmandaL Phelps, *Defence Science Technology Laboratory (Dstl), Salisbury, United Kingdom*

346. Unraveling the Mechanism of Action of the Broad-spectrum Antiviral Peptide Labyrinthopeptin A1
Merel Oeyen, Ph.D., *KU Leuven Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

347. Unveiling Antiviral Mechanisms of Plitidepsin by In-Silico Transcriptomic Analysis
Jose A. Lopez-Martin, M.D., Ph.D., *PharmaMar – Virology, Colmenar Viejo, Madrid, Spain*

348. Viral Capsids as Tools for Structural Biology
Petr *Škvára*, **Ph.D. Seeking**, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

349. Virucidal Drugs
Francesca Olgiati, M.S., *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

350. Efficacy and Safety of Zapnometinib in Hospitalized Adult Patients with COVID-19: A Randomized, Double-blind, Placebo-controlled, Multi-center, Phase 2 Trial (RESPIRE)
Stephan Stenglein, M.D., *Atriva Therapeutics GmbH, Germany*

352. Oral 4'-Fluorouridine Protects against Lethal Lassa virus Infection in Guinea Pigs and Can Rapidly Improve Clinical Signs When First Dose Administered During Peak Febrile Period of Disease
Jessica R. Spengler, D.V.M., Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*

353. Single Dose Mucosal Delivery of a Nipah VRP-Based Vaccine Confers Rapid Protection Against Lethal Disease
Stephen R. Welch, Ph.D., *CDC, Atlanta, Georgia, United States*

354. Evaluation of Remdesivir and Monoclonal Antibody m102.4 Combination Against Nipah virus in Human Primary-like Small Airway Epithelial Cells in vitro
Michael Lo, Ph.D., *US Centers for Disease Control and Prevention, Atlanta, GA, United States*

355. Development and Utilization of a Novel Minigenome and Recombinant Vesicular Stomatitis Virus Expressing Seoul Hantavirus Glycoprotein-based Assays to Identify Promising Anti-hantavirus Therapeutics
Punya Shrivastava-Ranjan, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*

356. A Comparative Analysis of Host Response and Inhibition Amongst Diverse Ebolavirus Species
Laura K McMullan, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

357V. Viral Replication and Host Immune Responses Early After Challenge in Mice Vaccinated with Crimean-Congo Hemorrhagic Fever Virus Replicon Particles
Teresa E. Sorvillo, Ph.D., *Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States*

358V. Atypical Mutational Spectrum of SARS-CoV-2 Replicating in the Presence of Ribavirin
Pilar Somovilla Crespo, Ph.D., *Centro de Biología Molecular Severo Ochoa (CBM-CSIC), Madrid, Spain*

359V. Broad Spectrum Inhibitors Targeting the SAM-binding Site of Viral Methyltransferases
Hongmin Li, Ph.D., *University of Arizona, Tucson, Arizona, United States*

360V. Frog Skin AMPs: Promising Antiviral Peptides
Annalisa Chianese, Ph.D., *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*

361V. Pre-Clinical Efficacy of Nanobodies as Passive Immunotherapeutics in the Golden Hamster Model of SARS-COV-2 Infection
Yu Cong, M.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*

362V. Screening of Peptides Designed on Schmallenberg Virus Glycoproteins Able to Inhibit the Viral Infection
Carla Zannella, Ph.D., *University of Campania Luigi Vanvitelli, Naples, Naples, Italy*

363V. SRI-44249, a Novel Anti-Influenza Lead Compound that is Active Against Influenza A Viruses and Targets RNA-Dependent RNA-Polymerase Function
Fahim Ahmad, Ph.D., *Southern Research, Birmingham, AL, United States*

364V. Targeting West Nile Virus Replication by Xanthine Inhibitors
Corinne Augelli-Szafran, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Omar Moukha-Chafiq, Ph.D., *Southern Research, Alabama, United States*

400. A Novel Approach to Bridge the Gap from Virology Research to New Antivirals
Andreas Kühbacher, Ph.D., *AiCuris Anti-infective Cures AG, Wuppertal, Germany*

401. Cell Culture Studies of the Barrier to Resistance of Broad-spectrum Antiviral Remdesivir against Hepatitis C Virus
Kuan Wang, Ph.D. Seeking, *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*

402. Combination of HBV Capsid/core assembly Modulators (CAMs) Leads to a Long-lasting Antiviral Effect in vitro
Julien Pronost, M.S., *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France*

403. Discovery of Small Molecule Antivirals Targeting Hepatitis B Virus Epsilon Element
Melissanne de Wispelaere, Ph.D., *Evotec ID (Lyon) SAS, Lyon, France*

404. Pharmacological Evaluation of N-hydroxypyridinediones to Support Optimization as HBV Ribonuclease H Inhibitors
Molly Woodson, Ph.D. Seeking, *St Louis University, Department of Molecular Microbiology and Immunology, St Louis, MO, United States*

405. HBV RNaseH inhibitors Can Decrease Capsid Accumulation and May Induce an Interferon Response in HBV Replicating Cells.
Molly Woodson, Ph.D. Seeking, *Saint Louis University, Saint Lous, MO, United States*

406. Hepatoma Cell Line Allowing Efficient Replication of Hepatitis B/C/D/E Viruses Can Be a Relevant Model for Drug Screening
Roxanne Fouille, Ph.D. Seeking, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*

407. High Activity of Remdesivir and Other Broad-spectrum Antivirals for the Treatment of Multidrug Resistant Hepatitis C Virus in vitro
Santseharay Ramirez, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*

408. Influence of Assembly Modulators on the Structure and Assembly Kinetics of Hepatitis B virus capsid
Kalouna Kra, Ph.D. Seeking, *Institut de Biologie Intégrative de la Cellule, CNRS, Université Paris-Saclay, CEA, GIF-SUR-YVETTE, Essonne, France*

409. Sofosbuvir as a Mild Mutagen for Hepatitis C Virus
Brenda Martínez-González, Ph.D. Seeking, *Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain*

410. Structure-Guided Engineering of Active Hepatitis B Virus Ribonuclease H
John Tavis, Ph.D., *Saint Louis University School of Medicine, Saint Louis, MO, United States*

411. The Combination of Bemnifosbuvir (BEM) and Ruzasvir (RZR), the HCV NS5b and NS5A Inhibitors, Demonstrates Potent In Vitro Synergistic Antiviral Activity and In Vivo Preclinical Safety Without Adverse Precautions
Nancy Agrawal, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*

412. Within-cell Dynamics of Wildtypem Virus-defective Genomes-RNA Satellites: A Mathematical Approach
Josep Sardanyés, Ph.D., *Centre de Recerca Matemàtica, Cerdanyola del Vallès, Barcelona, Spain*

413V. Novel Pyrrolopyrimidine Nucleoside Analogs as Anti-hepatitis B Virus Agent
Omar Moukha-Chafiq, Ph.D., *Southern Research, Birmingham, Alabama, United States*

414V. Systematic Mutagenesis Studies Reveal Amino Acid Residues Critical for HBeAg Biogenesis and Mechanism of Capsid Assembly Modulator Inhibition of HBeAg Secretion
Hui Liu, Ph.D., *Baruch S Blumberg institute, Doylestown, Pennsylvania, United States*

500. **27-hydroxycholesterol Inhibits Rhinovirus Replication in vitro and On Human Nasal and Bronchial Histocultures Without Selecting Viral Resistant Variants**
Matteo Costantino, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*

501. **A Broad-spectrum Small Molecule Anti-viral Targeting SARS-CoV-2 and Pandemic Influenza Viruses**
Gudepalya Renukaiah Rudramurthy, Ph.D., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*

502. **A High-throughput Discovery Platform Supporting Early and Advanced Antiviral Programs**
Miranda Nebane, Ph.D., *Southern Research, Birmingham, AL, United States*

503. **A Mutation in the Coronavirus nsp13-Helicase Confers Partial Remdesivir Resistance and Alters Enzymatic Activity**
Mark Denison, M.D., *Vanderbilt University Medical Center, Nashville, TN, United States*

504. **A Non-Excisable Nucleotide Analogue Active against SARS-CoV-2**
Ashleigh Shannon, Ph.D., *AMU, Marseille, France*

505. **A Novel Transgenic Mouse Model (R26AGP-hACE2 in B6 Mice) Expressing Human ACE2 for Studying the Pathogenesis of SARS-COV-2 Infection**
An-Yu Chen, M.S., *The Institute of Preventive Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China*

506. **A Reporter Cell Line for the Automated Quantification of SARS-CoV-2 Infection in Living Cells**
Yves Rouillé, Ph.D., *CNRS, Lille, France*

507. **Analysis of SARS-CoV-2 Variants from Patient Specimens in Nevada from October 2020 to August 2021**
Cyprian C. Rossetto, *University of Nevada, Reno School of Medicine, Reno, NV, United States*

508. **Anchimerically Activatable ProTide Inhibitors of Eukaryotic Translation Initiation Factor 4E (eIF4E) as Host-Directed Antivirals Against SARS-CoV-2**
Jacob A Smith, Ph.D. Seeking, *University of Minnesota-Twin Cities, Minneapolis, MN, United States*

509. **Antiviral Activity of a Metabolite from Hypericum perforatum L. Against Human Coronaviruses**
Imelda Raczkiewicz, M.S., *Center for Infection and Immunity of Lille (CIIL), Lille, France*

510. **Antiviral Activity of EIDD-1931 and Remdesivir Against SARS-CoV-2 Variants in 3D Mucociliary Tissue Models Consisting of Normal, Human-derived Tracheal/ Bronchial (EpiAirway) and Nasal (EpiNasal) epithelial Cells**
Kie Hoon Jung, Ph.D., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States*

511. Antiviral Development of Pan-coronavirus Small Molecules Targeting Intracellular Dynamics
Raphael Gaudin, Ph.D., *CNRS, Montpellier, France*

512. Antiviral Effect of 2-Deoxy-D-Glucose on Replication of Human Coronaviruses
Vitalii Kovtunyk, M.S., *G.ST Antivirals GmbH, Vienna, Austria*

513. Antiviral Properties of stilbene Dimers Obtained Bychemoenzymatic Synthesis Against Enveloped Viruses
Arnaud Charles-Antoine Zwygart, M.S., *University of Geneva, Geneva, Switzerland*

514. Apixaban, an Orally Available Anticoagulant, Inhibits SARS-CoV-2 Replication and its Major Protease in a Non-Competitive Way
Otavio Augusto Chaves, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*

515. Biochemical Characterization of Peptide-based Inhibitors of the Influenza Polymerase PA-PB1 Subunit Interaction
Michal Kral, Ph.D. Seeking, *Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic*

516. C6-Alkynyl-2,4-quinazolinedione-N-1-ribonucleoside Analogs and Their Phosphoramidates for the Inhibition of SARS-CoV-2
Vincent Roy, Ph.D., *ICAO, Orléans, France*

517. Characterization of Antibodies Against SARS-CoV-2 Capping Enzymes nsp10 and nsp14 and Their Use as a Tool in SARS-CoV-2 Research
Vladimira Horova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

518. Combination of the Parent Analogue of Remdesivir (GS-441524) and Molnupiravir results in a Markedly Potent Antiviral Effect in SARS-CoV-2 Infected Syrian Hamsters
Rana Abdelnabi, Ph.D., *Rega Institute, KU Leuven, Leuven, Belgium*

519. Curcuminoid Analogues Suppress Influenza A Virus Replication and Expression of Pro-inflammatory Cytokines and Interferons in A549 Lung Epithelial Cells by Inhibiting Multiple Retinoic Acid-inducible Gene-I (RIG-I)-mediated Pathways
Kong Yen Liew, Ph.D. Seeking, *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

520. Design and Synthesis of Trimeric Compounds as Fusion Inhibitors of Hemagglutinin of Influenza Virus
Sonia De Castro, Ph.D., *Instituto de Química Médica (CSIC), Madrid, Spain*

521. Development of New Benzofuran Derivatives as STING Agonists with Antiviral Activity
Annalaura Paulis, *Università degli studi di Cagliari, Cagliari, Italy*

523. EDP-235, a Potent, Once-daily, Oral Antiviral, Demonstrates Excellent Penetration into SARS-CoV-2 Target Tissues, with the Potential for Mitigation of Viral Rebound in COVID-19 Patients
Indy Zang, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*

524. EDP-235, an Oral, Once Daily, Ritonavir-Free, 3CL Protease Inhibitor for the Treatment of COVID-19: Results From Phase 1 Study in Healthy Subjects
Guy De La Rosa, M.D., *Enanta Pharmaceuticals, Watertown, MA, United States*

525. Enhanced Neutralization Escape and Fusogenicity of SARS-CoV-2 Omicron Subvariants
Shan-Lu Liu, M.D., Ph.D., *The Ohio State University, Columbus, Ohio, United States*

526. Evaluation of Molnupiravir and GS-441524 as Treatments for a Lethal Neurologic SARS-CoV-2 Infection in hACE2 Mice
Scott Gibson, Ph.D. Seeking, *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

527. Evaluation of the Nucleoside Analogs Antiviral Potential Against SARS-CoV-2
Anastasia L. Khandazhinskaya, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*

528. High Throughput Screen to Identify Non-Nucleoside Small Molecule Inhibitors of SARS-CoV-2 RNA-dependent RNA polymerase
Tessa Cressey, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*

529. Identification and preclinical development of kinetin as a safe error-prone SARS-CoV-2 antiviral able to attenuate virus-induced inflammation
Thiago Souza, Ph.D., *Fiocruz, Rio de Janeiro, Brazil*

530. Identifying the in cellulo Activity of Broad-spectrum Antivirals Against Serpentoviruses
Justin G. Julander, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, UT, United States*

531. In vitro and in vivo Characterisation of Respiratory Syncytial Virus (RSV) Inhibitors Using the Example of Presatovir
Pia Thommes, *Evotec UK, Alderley Park, United Kingdom*

532. In Vitro Antiviral Profile of AB-343, a Novel, Oral, Potent SARS-CoV-2 Mpro Inhibitor with Pan-coronavirus Activity
Nagraj Mani, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*

533. In vitro Characterization and Optimization of Antiviral Aerosol Therapy Against SARS-CoV-2 and Other Respiratory Viruses
Christin Müller, Ph.D., *Justus Liebig University, Institute of Medical Virology, Giessen, Germany*

534. In vitro Combinations of Ribavirin with Remdesivir or GS-441524 Result in Synergistic Inhibition of hPIV3 Replication
Yuxia Lin, *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

535. In Vitro Selection of SARS-CoV-2 Variants With Remdesivir or Molnupiravir Reduced Susceptibility
Carlota Fernandez-Antunez, M.S., *CO-HEP, Copenhagen, Denmark*

536. Localization of RNA-dependent RNA Polymerase from Picornaviruses In Cells Using Fluorescent NTPs and Synthetic Transporters
Barbora Landova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

537. Low Risk of Drug-Drug Interactions (DDIs) for Bemnifosbuvir (BEM) Based Upon In Vitro Metabolism and Transporter Interaction Studies
Alex Vo, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*

538. Mechanism of Action and Drug-resistance of Remdesivir, a Viral RNA Chemical Corruptor
Bhawna Sama, Ph.D. Seeking, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*

539. Mechanistic Characterization of Two Distinct Inhibitors of Coronavirus Nsp15 Endoribonuclease
Benjamin Van Loy, *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

540. Modulation of in vitro SARS-CoV-2 Infection by Stephania Tetrandra and Its Alkaloid Constituents
Stephen Polyak, Ph.D., *University of Washington, Seattle, WA, United States*

541. Molecular Scaffolds for Macro Domain Targeted Inhibition
Oney Ortega Granda, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France*

542. Morbidity and Mortality of SARS-CoV-2 Infections in PLWH in Uganda a Retrospective Cohort Study
Waiswa Moses, M.S., *Amani Children's Clinic, Kampala, Uganda*

543. Mung Bean Extract Inhibits Feline Coronavirus In Vitro
Ai-Ai Chou, B.S., *National Taiwan University, Taipei, Taiwan, Republic of China*

544. New Fleximer Aza/deaza Nucleoside Analogues: Anti SARS-CoV-2 Activity and Possible Targets
Elena Matyugina, Ph.D., *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia*

545. Nirmatrelvir Resistant SARS-CoV-2 Variants with High Fitness in an Infectious Cell Culture System
Yuyong Zhou, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Hvidovre, Denmark*

546. Novel Attachment Inhibitors of Human Parainfluenza 3
Gregory Mathez, M.S., *Institute of Microbiology, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland*

547. Novel SARS-CoV-2 nsp14 Inhibitors as Potential Antiviral Compounds
Radim Nencka, Ph.D., *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

548. Peruvian Amazonian Medicinal Plants With Antiviral Activities Against Human Coronavirus
Karin Séron, Ph.D., *Center for Infection and Immunity of Lille, Lille, France*

549. Pharmacokinetics and Metabolism of [14C]-bemnifosbuvir in Healthy Male Participants
Xiao-Jian Zhou, Ph.D., *Atea Pharmaceuticals, Inc., Boston, MA, United States*

550. Potent Phosphoramidate Prodrugs of the Remdesivir Parent Nucleoside GS 441524) for Intramuscular Injection
Rao V. Kalla, Ph.D., *Gilead Sciences, Foster City, California, United States*

552. SARS-CoV-2 Perturbs Polyamine Metabolism to Ensure Efficient Replication: Studies Using Plasmax Culture Medium
Alexander V. Ivanov, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*

553. Sars-Cov-2 Viruses with Cross-Resistance to 3CLpro Inhibitors Can Be Selected In Vitro, and Can Replicate and Transmit in a Hamster Model
Dirk Jochmans, Ph.D., *KU Leuven – Rega Institute, Leuven, Belgium*

554. Structure-guided Design of Protease-resistant, Lipopeptide Inhibitors of SARS-CoV-2
Ariel Kuhn, Ph.D., *University of Wisconsin – Madison, Madison, WI, United States*

555. Targeting the Host Cell Metabolism to Fight Respiratory Viral Infections
Anna-Dorothea Gorki, Ph.D., *G.ST Antivirals GmbH, Vienna, Austria*

556. Targeting the Interaction Between Host MASP-2 and the Viral N Protein as a Broad-spectrum therapeutic Approach for coronavirus Infections
Ben Flude, Ph.D. Seeking, *Swansea University, Swansea, United Kingdom*

557. Targeting the Main Protease to Develop the SARS-CoV-2 Antivirals
Wenshe Liu, Ph.D., *Texas A&M University, College Station, TX, United States*

558. Tracking SARS-CoV-2 Variants for Monoclonal Therapeutic Antibodies Evaluation
Franck Touret, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*

559. Treatment of EV-D68 Neurological Disease in IFNAR Mice with EIDD-1931 and Human Intravenous Immunoglobulin
Brett Hurst, Ph.D., *Utah State University, Logan, Utah, United States*

560. Trypthophan Derivatives Block SARS-CoV-2 Entry by Interfering the Interaction of ACE2 with the Viral Spike
María-Jesús Pérez-Pérez, Ph.D., *Instituto de Quimica Medica (IQM, CSIC), Madrid, Spain*

561. Umifenovir and Interferon -alpha-2b Are Broadly Effective Against SARS-CoV-2 Variants
Irina A. Leneva, Ph.D., *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*

562. USC-026 and USC-089a Suppress the Replication of SARS-CoV-2 in the Lungs of Syrian Hamsters and Mitigate Viral Pathogenesis More Effectively Than Remdesivir
Charles E. McKenna, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*

563. Use of Deep Learning In Design of New Antiviral Candidates Targeting Both Wild and Mutant Influenza A Virus
Hovakim Zakaryan, Ph.D., *Denovo Sciences Inc, Yerevan, Armenia*

564. β-D-N4-Hydroxycytidine (NHC), Active Ribonucleoside Analog of Molnupiravir, Impairs Viral RNA Synthesis and Recombination of SARS-CoV-2, MERS-CoV, and MHV
Laura Stevens, M.S., *Vanderbilt University Medical Center, Nashville, TN, United States*

566V. Comparison of SARS-CoV-2 Variants in a Transgenic hACE2-Mouse Model
E. Bart Tarbet, Ph.D., *Utah State University, Logan, Utah, United States*

568V. Impact of Baloxavir-resistant Influenza Virus Mutants on Viral Growth and Drug Susceptibility
Brady Hickerson, Ph.D., *FDA, Silver Spring, Maryland, United States*

569V. Novel Formulations of Remdesivir as Organic Salts and Ionic Liquids (OSILs) Show Improved in vitro Antiviral Activity Against SARS-CoV-2
Vanessa Correia, Ph.D., *National Health Institute Dr. Ricardo Jorge (INSA), Lisbon, Portugal*

570V. Substituted Phenyl Ethynyl Pyridine Carboxamides as Potent Inhibitors of SARS-CoV-2 Virus
Corinne Augelli-Szafran, Ph.D., *Southern Research, Birmingham, Alabama, United States*

571V. Susceptibility of Mammalian Cell Lines to Infection with H3N2 Influenza Virus and Differential Expression of ANP32A, an Acidic Leucine-Rich Nuclear Phosphoprotein
Babu L. Tekwani, Ph.D., *Infectious Diseases Department, Scientific Platforms, Southern Research, Birmingham, Alabama, United States*

600. A Host Kinase Inhibitor, VKT-034, Is Highly Effective Against Vacells and Human Skin Organ Culture
Jennifer Moffat, Ph.D., *SUNY Upstate Medical University, Syracuse, New York, United States*

601. Accumulation of Mutations in BKPyV Genome In Kidney Transplant Recipients Treated with Cidofovir
Olga Mineeva-Sangwo, Ph.D., *Department of Microbiology, Immunology and Transplantation, Katholieke Universiteit Leuven, Leuven, Belgium*

602. An HSV-1 DNA Polymerase Multidrug Resistance Mutation Identified in an HSCT Recipient Confirmed by CRISPR/Cas9-mediated Gene Editing
Hanna H. Schalkwijk, *Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

603. Broad Anti-DNA Virus Activity of Novel Cyclic Amino Acid Phoshonamidate Prodrugs of Acyclic Nucleoside Analogues
Elisabetta Groaz, Ph.D., *Rega Institute, Medicinal Chemistry, Leuven, Belgium*

604. Differences in the Pharmacokinetics of Tyrosinamide (USC-087) and Homoserinamide (USC- 093) Prodrugs of (S)-HPMPA Are Responsible for the Lower Toxicity of USC-093 in the Syrian Hamster Model of Adenovirus Infection
Karoly Toth, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*

605. Effect of Castalagin Against HSV-1 Infection In Mice
Adelina Stoyanova, Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

606. Epstein-Barr virus-associated Posttransplant Lymphoproliferative Disorders: Providing An Inside Into the Genetics and Biology of EBV
Martyna Pociupany, Ph.D. Seeking, *KU Leuven, Leuven, Belgium*

607. Filociclovir is a Potent Inhibitor of Human Adenovirus F41 and a Strong Candidate for Treating Pediatric Adenovirus Infections
Islam Hussein, Ph.D., *Microbiotix, Worcester, United States*

608. HSV-1 Chromatin is Enriched in Highly Dynamic Histone Variant H2A.B in Replicating and Transcribed Viral DNA
Arryn Owens, B.S. Seeking, *Cornell University, Ithaca, NY, United States*

609. The Mechanism of Action of Filociclovir against Human Adenovirus is Different and Distinct from its Action against Human Herpes Viruses
Sydney Blackmer, B.S., *Drake University College of Pharmacy and Health Sciences, Des Moines, IA, United States*

610. USC-150, A Homoserinamide Oral Prodrug of Cidofovir, Prevents Lethal HAdV-C6 Infection in a Humanized Immunosuppressed Syrian Hamster Model
Samantha B. Riemann, B.S., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*

611. USC-374, A Novel Prodrug of HPMPA, Suppresses the Replication of Human Adenoviruses In vitro and in vivo and Protects Hamsters from Lethal HAdV-C6 Infection
Charles E. McKenna, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*

612V. Characterization of Novel, Potential Antiviral Resistance Mutations in the Monkeypox Virus Replication Complex in the 2022 Outbreak
Benjamin Liu, M.D., Ph.D., *Children's National Hospital, George Washington University, Washington, DC, United States*

613. Infectivity and Replication Inhibition Effect of 5-fluorouracil on Herpes Simplex Virus type-1 Associated with Mutations in Thymidine Kinase Gene
Ahmed Eisa Elhag Ibrahim, D.V.M., Ph.D., *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*

614V. Novel Latency-disrupting Anti-herpesvirals & PROTACs Against KSHV
Aylin Berwanger, *Helmholtz-Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany*

700. Antivirally Active Dialkyl-Nucleoside Diphosphate and Triphosphate Prodrugs
Xiao Jia, Ph.D., *University of Hamburg, Hamburg, Germany*

701. C-2 Alkylated Tryptophan Derivatives, Highly Potent Entry Inhibitors of Enterovirus A71 Clinical Isolates
Ana Rosa San Felix, Ph.D., *Instituto de Química Médica, Spanish Research Council (CSIC), MADRID, Madird, Spain*

702. Characterization of Brazilian HIV-1 Near Full-Length Proviral Genomes from Patients under Successful First-Line Antiretroviral Therapy
Ornella Botelho, M.S., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*

703. Longitudinal Evaluation of Archived HIV-1 Proviral Epitopes with High Affinity to Circulating HLA Class I Alleles as Potential Tools for an HIV Therapeutic Vaccine
Ornella Botelho, M.S., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*

704. Longitudinal Evolution of HIV-1 Drug Resistance Mutations Within Proviral Quasispecies of Patients Under Successful Antiretroviral Therapy
Brunna Alves, Ph.D., *INCA, Rio de Janeiro, Rio de Janeiro, Brazil*

705. S-Acyl-Benzamide Derivatives as HIV Inactivators
Marco Robello, Ph.D., *National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, United States*

706. Setting Up A Drug-discovery Platform for New HTLV-1 Antivirals
Clément Jacques François Heymann, Ph.D. Seeking, *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*

707V. Discovery, Mechanistic Investigations and Crystallographic Study of Benzopyrimidinone-bearing Phenylalanine Derivatives as Novel HIV-1 Capsid Modulators
Shujing Xu, Ph.D., *Shandong University, Jinan, Shandong Province, China*

800. Strigolactones as Broad-spectrum Antivirals Against β-coronaviruses Through Targeting the Main Protease Mpro
Greta Bajetto, M.S., *University of Piemonte Orientale, Center for Translational Research on Autoimmune and Allergic Disease-CAAD,, Novara, Italy*

801. Novel Benzodiazepine Antivirals Selectively Active against Yellow Fever Virus with a GABAA Receptor Refractory Property
Yanming Du, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

802. Evaluation of in Vitro Selected Nirmatrelvir Resistant SARS-COV-2 in A549-ACE2 Cells
Hussin Rothan, Ph.D., *Pfizer INC, NY, United States Minor Outlying Islands*

803. **SARS-CoV-2 Nirmatrelvir Resistance Selection in VeroE6-Pgp-KO Cells**
Irina Yurgelonis, M.S., *Pfizer, Pearl River, NY, New York, United States*

804. **Murine Hepatitis Virus (MHV) Mutations Selected in vitro by Nirmatrelvir, An Oral Coronavirus Main Protease (Mpro) Inhibitor**
Irina Yurgelonis, M.S., *Pfizer, Pearl River, NY, New York, United States*

805. **Nirmatrelvir, An Orally Active Mpro Inhibitor, Is A Potent Inhibitor of SARS-CoV-2 Variants of Concern**
DevendraK Rai, Ph.D., *Pfizer Worldwide Research, Development & Medical, Pearl River,, New York, United States*

806. **The 5'UTR of HCoV-OC43 Adopts a Topologically Constrained Structure to Intrinsically Repress Translation**
Barrington D. Henry Jr, Ph.D. Seeking, *Case Western Reserve University, Cleveland, OH, United States*

807. **Stabilization of RNA G-quadruplexes in the SARS-CoV-2 Genome Inhibits Viral Infection via Translational Suppression**
Moon Jung Song, Ph.D., *Korea University College of Life Sciences and Biotechnology, Seoul, South Korea*

808. **Exoribonuclease Activity As A Validated Target to Fight Arenaviruses and Coronaviruses Infections**
Karine Alvarez, Ph.D., *AFMB UMR 7257, CNRS, AMU, Marseille, France*

809V. **Antiviral Activity of Catechol and Its Derivatives Against Epstein-Barr Virus**
Min Sun Park, B.S., *Korea University, Seoul, South Korea*

001. The Quasispecies Challenge: In Search of Antiviral Synergisms with Lethal Mutagens

Esteban Domingo, Ph.D., *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain*

Abundant experimental evidence has shown that populations of replicating RNA viruses consist of myriads of mutant genomes that vary in frequency. They are termed viral quasispecies because their dynamics fits the theoretical quasispecies concept, applied to finite replicon populations. Genomes that participate in quasispecies dynamics may include mutations that confer resistance to antiviral agents. Therefore, replicating viral populations represent a moving target for antiviral interventions. Antiviral resistance mutations arise stochastically and can be selected in the presence of antiviral agents, resulting in treatment failure. High replicative fitness is also a determinant of antiviral resistance. Several strategies are under investigation to avoid selection of antiviral-resistant mutants. Our efforts have been focused on turning the error threshold concept of quasispecies theory into an antiviral design. Viruses do not maintain their infectivity if they mutate above an error threshold value. Enhanced error rates are achieved by nucleotide analogues that are incorporated into viral RNA. They exert their activity via at least three mechanisms: lethal defection, mutational biases, and overt lethality. Ribavirin, favipiravir and molnupiravir include lethal mutagenesis as part of their antiviral mechanism. At least 25 different RNA viruses have proven susceptible to lethal mutagenesis. Results with hepatitis C virus and SARS-CoV-2 will be summarized, with emphasis on prospects for synergistic antiviral combinations. Benefits and limitations of lethal mutagenesis for the control of established and emergent viral pathogens will be presented.

002. Addressing Viral Infections In Neglected Patient Populations: The Drugs for Neglected Diseases Initiative's Efforts In HIV, HCV, COVID-19 and Pandemic Preparedness, and Dengue

Laurent Fraisse, Ph.D., *Drugs for Neglected Diseases initiative, Geneva, Genève, Switzerland*

Drugs for Neglected Diseases initiative (DNDi) is applying its expertise in developing treatments for neglected tropical diseases to neglected populations affected by viral infections. For children living with HIV (CLHIV), previous paediatric treatment options were poorly adapted, bitter-tasting syrups. DNDi and partners developed strawberry-flavoured 4-in-1 granules, a safe and effective alternative. For CLHIV with TB, a treatment super-boosted with ritonavir was developed to overcome drug-drug interactions with rifampicin-based TB therapy. Access to affordable direct-acting antivirals for HCV is limited in many low- and middle-income countries (LMIC). DNDi and partners developed ravidasvir as part of a highly effective, simple-to-use, affordable combination treatment. To reduce the burden on LMIC health systems and find field-adapted solutions, DNDi launched the ANTICOV consortium, a multi-country, adaptive platform trial in Africa, to identify treatments for mild/moderate COVID-19 and worked with the Access to COVID-19 Tools Accelerator (ACT-A) to review candidates for clinical testing. DNDi joined the 'COVID Moonshot' project and the new AI-driven Structure-enabled Antiviral Platform (ASAP) to build an antiviral discovery pipeline for pandemic preparedness. DNDi is bringing together endemic country partners in an alliance to develop affordable, adapted treatments for dengue that prevent progression to severe disease. We need innovation in therapeutics and formulations targeted to the healthcare needs of neglected populations worldwide. Access is critical to successful delivery of treatments and must be integrated into the research and development of new antivirals.

003. Discovery Science for the Cure of Hepatitis B Virus Infection

Fabien Zoulim, M.D., Ph.D., *INSERM, Lyon University, Hospices Civils de Lyon, Lyon, France*

Current treatment of chronic hepatitis B (CHB) with nucleoside analogues can suppress HBV replication efficiently. However, lifelong therapy is needed in the majority of patients because of the persistence of HBV cccDNA in infected cells. The major barriers to HBV cure include the reservoirs for HBV replication, the impaired innate immunity, and the exhausted adaptive immune responses against HBV. Based on hepatocyte culture systems and small animal models, investigations have allowed to unravel the fundamental mechanisms of viral persistence, identify novel immune and viral targets, and discover new agents to cure the infection. Translational research is performed in CHB patients to identify novel biomarkers to assist the clinical development of new drugs, and to identify correlates of cure. Several promising combination strategies are currently evaluated in clinical trials comprising : i) direct acting antivirals: viral entry inhibitors, capsid assembly modulators, nucleic acid polymers, ii) drugs targeting viral RNAs and viral antigen expression: siRNA and anti-sense oligonucleotides (ASO), iii) boosters of immune responses: TLR7/8 agonists, immune check point inhibitors: PD1 / PDL1 blockade, iv) therapeutic vaccines to stimulate HBV specific B and/or T cells.

Many of these strategies are already in phase II trials, and ASO have entered phase III. Promising approaches are also explored to directly target cccDNA for degradation or for silencing, or to redirect T cells to infected hepatocytes. Altogether, the HBV cure field has entered a very exciting time with the hope of obtaining increasing rates of HBV cure through incremental clinical development.

004. Preparing for Tomorrow's Pandemics, Today Through the Development of Broad-spectrum Antivirals

Timothy P. Sheahan, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States*

Coronaviruses (CoV) are prone to emergence, the spillover from one host to another to cause new disease. Novel CoV emergence has occurred at least five times over the past 20 years and we are currently experiencing the consequence with the COVID-19 pandemic. Given this trend, it is likely that CoV emergence will continue in the future. To maximize pandemic preparedness, potent broadly active antiviral therapies are needed to treat current and future emerging CoVs. To address this, we have created multiple in vitro and in vivo models of CoV replication and disease within which to assess antiviral activity and efficacy. Prior to the COVID-19 pandemic, our team used these tools to assess the therapeutic potential of remdesivir and molnupiravir to treat emerging CoV infections. These data, among others, helped position these small molecules for testing in humans with COVID-19. With SARS-CoV-2 specific tools, we continue with this strategy to provide pre-clinical data for antiviral therapies of multiple modalities. Future work is needed to develop combination antiviral therapy that diminishes the potential for antiviral resistance and ameliorates pathogenic components of the host response. Prior to the COVID-19 pandemic, there were no approved vaccines or therapies to treat any human CoV infection but now we have multiple approved vaccines and antiviral therapies. This collective effort is a testament to the power of antiviral research which has increased our preparedness for future emerging CoV and created a roadmap for antiviral rapid response for future emerging viral diseases.

005. Development of an Orally Available Antiviral Drug for Yellow Fever

Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Fuxuan Wang, Ph.D., *Baruch S. Blumberg Institute, PA, United States*
Zhao Gao, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Lin Zhang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Sumangala Darsandhari, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Lauren Griffith, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Abbie E. Weight, B.S., *Utah State University, Logan, UT, United States*
Justin Julander, Ph.D., *Utah State University, Logan, UT, United States*
Yanming Du, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Ju-Tao Guo, M.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

In a cell-based high throughput screening, we identified a benzodiazepine compound BDAA that specifically inhibits yellow fever virus (YFV) replication, with resistant mutation mapped to YFV nonstructural protein 4B (NS4B). Structure-activity-relationship study has led to the identification of lead candidates, which showed improved druggable properties and 100% therapeutic protection against lethal YFV infection in hamster model. In support of preclinical development of this family of antiviral compounds, we performed extensive mechanistic studies. BDAA treatment induces dramatic ultrastructure alteration of viral replication organelles (ROs) and exposure of dsRNA in cytoplasm of virus-infected cells, which are found to be associated with enhancement of YFV-induced inflammatory cytokine response. These results support a model that BDAA's interaction with NS4B impairs the integrity of YFV RO, which not only directly abrogates viral genome replication, but also promotes viral replication intermediates releasing from RO to activate cytosolic RNA sensing pathways. Indeed, BDAA directly inhibits nascent YFV RNA synthesis in cell culture in a 5-ethynyl uridine incorporation experiment as well as in an endogenous polymerase reaction system using isolated ROs. Furthermore, we demonstrated that BDAA treatment activates three major dsRNA recognizing pathways, RLR, PKR and OAS-RNase L, in YFV-infected cells. Taken together, BDAA primarily hits the YFV RO and executes an unprecedented multi-mode antiviral action which may collectively lead to rapid-acting and potent inhibition of viral replication in vivo. MOA will be further examined in the forward preclinical/clinical studies.

006. A Pan-serotype Antiviral In Early Clinical Development for the Prevention and Treatment of Dengue: A Journey From Discovery to Clinical Development Driven by Public-private Partnerships

Marnix Van Loock, Ph.D., *Global Public Health R&D, Janssen Pharmaceutica NV, Beerse, Belgium*
Olivia F. Goethals, *Global Public Health R&D, Janssen Pharmaceutica NV, Belgium*
Natalia V. Voge, *Global Public Health R&D, Janssen Research & Development, LLC, Spring House, Pennsylvania, United States*
Bart Kesteleyn, *Janssen Research & Development, Janssen Pharmaceutica NV, Beerse, Belgium*
Patrick Chaltin, *Centre for Drug Design and Discovery (CD3), KU Leuven, Bioincubator 2 and Cistim Leuven vzw, Bioincubator 2, Leuven, Belgium*
Timothy Jinks, *Wellcome Trust, London, United Kingdom*
Tine De Marez, *Global Public Health R&D, Janssen Research & Development, LLC, Titusville, New York, United States*
Anil Koul, *Global Public Health R&D, Janssen Pharmaceutica NV, Beerse, Belgium*
Ruxandra Draghia-Akli, *Global Public Health R&D, Janssen Research & Development, LLC, Spring House, Pennsylvania, United States*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*

While progress has been made in fighting diseases disproportionally affecting underserved populations, unmet medical needs persist for many neglected tropical diseases. Different parties are contributing in several ways to address this imbalance. The Global Public Health initiative from Johnson & Johnson was launched in late 2014 to discover, develop, and deliver transformational solutions to tackle changing health challenges facing people across the world in the 21st century. The World Health Organization has encouraged strong public-private partnerships in public health, and a number of public and private organizations have set an example demonstrating a strong commitment to combat these diseases. We exemplify the role of a public-private partnership in research and development by the journey of our dengue antiviral molecule that is now in clinical development. We detail the different steps of drug discovery and development and outline the contribution of each partner to this process. Years of intensive cross-sector collaboration resulted in the identification of two antiviral compounds, JNJ-A07 and JNJ-1802, the latter advancing to clinical development.

007. The mechanism of RNA capping by SARS-CoV-2

Vincent Tagliabracci, Ph.D., *HHMI/UT Southwestern, Dallas, TX, United States*
Gina Park, *UT Southwestern, United States*
Adam Osinski, *UT Southwestern, Dallas, United States*
Genaro Hernandez, *UT Southwestern, Dallas, United States*
Jennifer Eitson, *UT Southwestern, Dallas, United States*
Abir Majumdar, *UT Southwestern, Dallas, United States*
Krzysztof Pawlowski, *UT Southwestern, Dallas, United States*
Marco Tonelli, *University of Wisconsin-Madison, Madison, United States*
Katie Henzler-Wildman, *University of Wisconsin-Madison, Madison, United States*
Yang Li, *UT Southwestern, Dallas, United States*
Zhe Chen, *UT Southwestern, Dallas, United States*
John Schoggins, *UT Southwestern, Dallas, United States*

The RNA genome of SARS-CoV-2 contains a 5′ cap that facilitates the translation of viral proteins, protection from exonucleases and evasion of the host immune response. How this cap is made in SARS-CoV-2 is not completely understood. Here we reconstitute the N7- and 2′-O-methylated SARS-CoV-2 RNA cap (7MeGpppA2′-O-Me) using virally encoded non-structural proteins (nsps). We show that the kinase-like nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain of nsp12 transfers the RNA to the amino terminus of nsp9, forming a covalent RNA–protein intermediate (a process termed RNAylation). Subsequently, the NiRAN domain transfers the RNA to GDP, forming the core cap structure GpppA-RNA. The nsp14 and nsp16 methyltransferases then add methyl groups to form functional cap structures. Structural analyses of the replication–transcription complex bound to nsp9 identified key

interactions that mediate the capping reaction. Furthermore, we demonstrate in a reverse genetics system that the N terminus of nsp9 and the kinase-like active-site residues in the NiRAN domain are required for successful SARS-CoV-2 replication. Collectively, our results reveal an unconventional mechanism by which SARS-CoV-2 caps its RNA genome, thus exposing a new target in the development of antivirals to treat COVID-19.

009. Structural Studies Facilitate Antiviral Drug Development Targeting the SARS-CoV-2 Main Protease and Variants of concern

Joanne Lemieux, Ph.D., *University of Alberta, Edmonton, AB, Canada*
Jimmy Lu, Ph.D. Seeking, *University of Alberta, AB, Canada*
Elena Arutyunova, Ph.D., *University of Alberta, Edmonton, AB, Canada*
Amelia Chen, Ph.D. Seeking, *University of Alberta, Edmonton, AB, Canada*
Jegan Iyyathurai, Ph.D., *University of Alberta, Edmonton, AB, Canada*
Bing Bia, Ph.D., *University of Alberta, Edmonton, AB, Canada*
Muhammad Bashir Khan, Ph.D., *University of Alberta, Edmonton, AB, Canada*
JamesA. Nieman, Ph.D., *University of Alberta, Edmonton, AB, Canada*

Despite progress in vaccine development, antivirals targeting SARS-CoV-2 are needed to help combat infection in regions where vaccines are not available or for those who are immunocompromised. Proteases cleave peptide bonds of a very specific sequence making them strong drug targets. Antivirals that target proteases are already used clinically to treat HIV and Hepatitis C virus, and recently the release of PaxlovidTM from Pfizer demonstrated the druggability of the SARS-CoV-2 main protease (Mpro). PaxlovidTM contains both an Mpro inhibitor, nirmatrelvir, as well as CYP inhibitor, ritonovir, and overall has a high pill load, suggesting this combination may not work for all patients. We have developed peptidomimetic inhibitors against SARS-CoV-2 to prevent the main protease from cleaving the viral polypeptide and subsequent viral replication in cells. Our early studies focused on the re-purposing of the feline coronavirus protease inhibitor, GC376. X-ray crystallography revealed the mechanism of inhibition, and has helped the optimisation of new derivatives. During optimisation, new peptidomimetic derivatives were designed and variations were made with both the warhead region and subsite-binding region of the compounds. Structural studies indicate the mechanism of increased potency. Our lead compounds have low nanomolar IC50 values and submicromolar EC50 values, which excel the in vitro results of nirmatrelvir (PaxlovidTM). Moving forward, these inhibitors have been tested with variant proteases, to measure efficacy. Animal studies in animals to determine pharmacokinetics and efficacy are underway in preparation for clinical trials.

010. Fast and Efficient Elimination of Latent and Lytic CMV Infection

Thomas N. Kledal, Ph.D., *Synklino A/S, Copenhagen, Copenhagen, Denmark*

CMV infection is the cause of significant morbidity in immunocompromized patients. Transmission of latent CMV via transplantation and post-transplant viral reactivation is extremely prevalent and results in inferior long-term outcomes, including servere disease and re-hospitalization, increased cost of transplantation and increased mortality. While current standard of care (SOC) offer some protection via inhibition of lytic replication, they have no effect on the latently infected cell reservoir. SYN002 is a novel biopharmaceutical in development for CMV in transplantation. SYN002 is a fusion toxin protein, targeting the CMV encoded chemokine receptor US28 which is expressed during both latency and lytic infection. SYN002 is selectively internalized into the infected cells, and drive the cells to undergo apoptosis via inhibition of elongation factor 2. SYN002 selectively, efficiently and potently eliminates both lytic and latently infected cells in vitro, including naturally latently infected monocytes significantly reducing reactivation. The ability to target latency combined with the fast mechanism of action opens for novel and innovative therapeutic strategies, including targeting CMV in organs ex vivo prior to tranplantation. In living human lungs in Ex Vivo Lung Perfusion, 6 hours of SYN002 therapy delivered through the pulmonary artery markedly attenuated CMV reactivation with no acute toxic events based on physiology and quantification of cytokines. Our studies demonstrate that SYN002 fast and efficiently can eliminate both latently and lytic infected cells, and that SYN002 holds the promise to change the current treatment paradigm being.

011. Challenges in Anti-Hepatitis D Virus Research: Insights From Preclinical and Clinical Studies

Maura Dandri, Ph.D., *University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

The hepatitis delta virus (HDV) is the causative agent of chronic hepatitis Delta (CHD). HDV is a defective RNA virus that relies on HBV envelope proteins to release infectious particles. Due to its compact genomic organization and lack of its own polymerase, HDV offers very few therapeutic targets. PegIFNα is commonly used for CHD treatment but its MoA in HDV-infected cells remains unclear and responsiveness is limited. The limited availability of cloned HDV strains and infection models have hindered understanding of the mechanisms involved in HDV persistence and therapy development. Studies in HBV/HDV infected humanized mice showed that HDV triggers the enhancement of ISGs, chemokines and genes involved in antigen presentation. Such ability to boost antigen presentation may contribute to liver inflammation. Intriguingly, and in strong contrast with HBV, HDV can disseminate also through cell division, a feature contributing to viral persistence. Remarkably, HDV appears more sensitive to IFN in dividing cells in vitro, although the impact of IFN on hepatocyte proliferation in vivo needs further investigation. Bulevirtide (BLV, Hepcludex®) is the only approved treatment for CHD in Europe. Intrahepatic analyses performed in paired liver biopsies (BL + 48w) from the clinical trials MYR203 and MYR301 showed a strong dose-dependent decline of intrahepatic HDV markers. Moreover, HDV decline was associated with a decrease in inflammatory gene expression, indicating an improvement in liver inflammation. In combination with pegIFNα, BLV appears to augment the antiviral efficacy. However, the underlying mechanisms of this synergy needs further investigation in preclinical models.

012. Structural Mechanism of Drug Resistance to L-nucleosides Conferred by the HIV-1 Reverse Transcriptase M184V Mutation

Eric Lansdon, Ph.D., *Gilead Sciences, Foster City, California, United States*

Emtricitabine (FTC) and lamivudine (3TC) are widely used nucleoside reverse transcriptase inhibitors (NRTIs) in antiretroviral therapy for HIV. Both drugs contain an oxathiolane ring with unnatural (-)-stereochemistry (L-nucleoside) as a mimic of ribose found in natural deoxynucleosides. Treatment with FTC or 3TC primarily select for HIV-1 Reverse Transcriptase (RT) M184V/I resistance mutations that are characterized by decreased incorporation of these NRTIs. Pre-steady state kinetic analysis reveals that active metabolites (-)-FTC-TP and (-)-3TC-TP have higher binding affinities for wild-type (WT) RT but slower incorporation rates than the natural substrate dCTP. The ternary crystal structures of (-)-FTC-TP and (-)-3TC-TP corroborate kinetic results demonstrating that their oxathiolane sulfur orients toward the DNA primer 3′-terminus and the triphosphate exists in two different binding conformations which likely contribute to their slower incorporation rate. Introduction of the M184V mutation shows a significant loss (>800-fold) in selectivity factor for the L-nucleotides compared to dCTP. The structure of M184V RT with (-)-FTC-TP illustrates how the mutation repositions the oxathiolane ring and induces a shift of the triphosphate into a conformation that hinders L-nucleotide binding. Taken together, the combined kinetic analysis and structural studies provides a comprehensive explanation for inhibiting WT HIV-1 RT by (-)-FTC-TP and (-)-3TC-TP and drug resistance caused by the M184V mutation.

013. Fine-Tuning Prodrugs of Acyclic Nucleoside Phosphonates

Zlatko Janeba, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*

The application of prodrugs has become an important strategy in drug design and development. Currently, about 10% of all marketed drugs worldwide are considered to be in the form of a prodrug (Nat. Rev. Drug Discov. 17: 559–587, 2018). Nucleotide analogues represent the key group of biologically relevant compounds where prodrug strategies have been studied extensively and where various types of masking groups have been introduced in order to mask the phosphate or phosphonate moiety. Recently, we have reported (J. Med. Chem. 64: 16425–16449, 2021) new prodrugs bearing tyrosine derivatives instead of the phenol moiety present in standard ProTides, e.g. in FDA-approved tenofovir alafenamide fumarate (TAF). In human lymphocytes, the most efficient tyrosine-based prodrug reached single-digit picomolar EC50 values against HIV-1 and nearly 300-fold higher selectivity index (SI) compared to TAF. In human hepatocytes, the most efficient prodrugs exhibited subnanomolar EC50 values for HBV and up to 26-fold higher SI compared to TAF. The reason for the outstanding potency of the prodrugs was investigated, nevertheless, their microsomal stability remained considerably lower compared to TAF. The promising biological data provided a strong foundation for further development of ANP prodrugs as highly potent antivirals and further studies are in progress, which aim both at increasing the microsomal stability of the prodrugs and at structural simplification of the masking groups.

014. Human Metapneumovirus: New Insights, New Mechanisms, New Targets

Rebecca Ellis Dutch, Ph.D., *University of Kentucky College of Medicine, Lexington, Kentucky, United States*
Cheng-Yu Wu, *University of Kentucky College of Medicine, KY, United States*
Chase Heim, *University of Kentucky College of Medicine, Lexington, KY, United States*
Carole Moncman, *University of Kentucky, Lexington, KY, United States*
Rachel Fearns, *Boston University School of Medicine, Boston, MA, United States*

Human metapneumovirus (HMPV) is a non-segmented, negative strand RNA virus that is a major cause of respiratory tract infections in infants, the elderly, and the immunocompromised. Though HMPV was identified in 2001, there are currently no FDA approved antivirals or vaccines available, and many questions remain about its infection processes. To dissect the infection process at the molecular level, paving the way for antiviral development, we have assessed key steps in viral replication and spread. We have shown that the viral replication centers, termed inclusion bodies (IBs), require actin dynamics for formation, and represent a class of phase-separated regions which change over the course of infection. In addition, our unpublished work shows that HMPV infection affects key steps in nucleotide biosynthesis and alters nucleotide levels, potentially regulating viral transcription and replication. Finally, we show that IBs can be directly moved from one cell to another via virus-induced connections between cells, identifying a new means of viral spread. Together, these findings provide new insights into the life cycle of this important respiratory virus and inform future work on antiviral development.

015. Inhibitors of the Spike Protein of Severe Acute Respiratory Syndrome Coronavirus 2

Sean P.J. Whelan, Ph.D., *Department of Molecular Microbiology, School of Medicine, Washington University in St. Louis, St. Louis, MO, United States*
Zhuoming Liu, *Department of Molecular Microbiology, School of Medicine, Washington University in St. Louis, MO, United States*

We developed and validated as a surrogate for SARS-CoV-2 entry a chimeric vesicular stomatitis virus that depends upon spike protein for infection of cells. The spike gene of Wuhan-1 strain and the subsequent variants including D614G, B.1.1.7, BA.1, BA.2, BA.5, BQ.1.1, XBB.1 were engineered into the VSV genome in place of the endogenous glycoprotein gene. Using panels of neutralizing monoclonal antibodies, multivalent minibinders and soluble receptor decoys, including those in clinical use or development, we selected variants resistant to inhibition. This work has yielded a library of over 360 different escape variants that also identifies critical positions in S associated with resistance to specific inhibitors and establishes fitness of different S mutations. Knowledge of such escape, coupled with sequence analysis of viruses circulating in humans, helps inform use of licensed therapeutic antibodies. Our mutation analysis also help study viral entry, antigenicity, immunogenicity and envelope protein evolution.

016. Single-domain Antibodies to Control Respiratory Viruses

Xavier Saelens, Ph.D., *VIB-UGent Center for Medical Biotechnology; Department of Biochemistry and Microbiology, Ghent, Belgium*

Single-domain antibodies are very versatile tools to study and interfere with virus entry pathways. We have described single-domain antibodies directed against influenza virus membrane proteins, the fusion protein of human respiratory syncytial virus, and spike of SARS-CoV-2 with broad in vitro and in vivo antiviral activity. Such single-domain antibody-based biologicals represent much needed drug candidates to prevent and treat disease caused by these and other respiratory viruses, in particular for immune-compromised populations with low vaccine responses. The presentation will highlight how a SARS-CoV-2 receptor-binding domain (RBD)-specific single-domain antibody was rapidly engineered into a stable anti-COVID-19 biological with excellent manufacturability. In addition, newly discovered, single-domain antibodies directed against the spike of SARS-CoV-2 with broad virus-neutralizing activity will be presented. Our findings also highlight that single-domain antibodies are promising candidate therapeutics to control respiratory viruses by targeting unusual epitopes.

017. A Novel SARS-CoV-2 Inhibitor Targeting the Membrane Protein With Activity in a SCID Mouse Model

Manon Laporte, Ph.D., *KU Leuven, Leuven, Belgium*
Dorothée Bardiot, *CISTIM, Belgium*
Dirk Jochmans, Ph.D., *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Philippe Arzel, *Belgium*
Mohamed Koukni, *Belgium*
Rana Abdelnabi, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Steven De Jonghe, Ph.D., *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Karolien Castermans, *Belgium*
Pieter Leyssen, Ph.D., *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Arnaud Marchand, *CISTIM, Leuven, Belgium*
Patrick Chaltin, *Centre for Drug Design and Discovery (CD3), KU Leuven, Bioincubator 2 and Cistim Leuven vzw, Bioincubator 2, Leuven, Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*

The SARS-CoV-2 Membrane glycoprotein (M) is the main component of the viral envelope. Being highly conserved and having multiple functions in the viral life cycle, the M protein is an attractive antiviral target. We report a novel compound class targeting SARS-CoV-2 M that was identified through a high throughput phenotypic screening effort. Following hit-optimization, compounds were obtained that block replication of ancestral SARS-CoV-2 and all variants of concern in the submicromolar range (EC50≤ 100 nM) in CPE based assays using VeroE6 and A549-ACE2-TMPRSS2 cells. Broader antiviral activity against SARS-CoV-1 was observed, but there is no activity against MERS or the α-coronavirus HCoV-229E . Time of drug addition assays indicate a late mechanism of action. In agreement with this, no activity was found in pseudovirus entry assays, nor protease or replicon assays. In parallel, in vitro resistance selection was performed and mutations in the M protein were identified. One specific substitution (P132S) resulted in a >10-fold shift in EC50. Finally we also demonstrated antiviral activity in vivo for an optimized hit (CIM-834) with favorable target tissue distribution. Oral treatment of SARS-CoV-2 (beta variant) infected SCID mice with CIM-834 resulted in a complete reduction of infectious virus titers in the lungs 3 days post-infection (50 mg/kg CIM-834 and 50 mg/kg ritonavir) . In conclusion, we present a novel class of coronavirus inhibitors with potent activity against SARS-CoV-2 in vitro and in an animal infection model. Elucidation of the precise mechanism of action and targeting of the M protein is currently being further explored.

018. Biochemical and Structural Insights Into SARS-CoV-2 Polyprotein Processing by Mpro: Implications for Developing Novel Antiviral Strategies

Ruchi Yadav, M.S., *Rutgers University, New Brunswick, Piscataway, NJ, United States*
Valentine V. Courouble, *The Scripps Research Institute, Jupiter, FL, United States*
Sanjay K. Dey, *Rutgers University, New Brunswick, Piscataway, NJ, United States*
Jerry Joe Harrison, *University of Ghana, Legon, Ghana*
Jennifer Timm, *Rutgers University, New Brunswick, Piscataway, NJ, United States*
Jesse B. Hopkins, *Illinois Institute of Technology, Chicago, IL, United States*
Ryan Slack, *Emory University School of Medicine, Atlanta, GA, United States*
Stefan G. Sarafianos, *Emory University School of Medicine, Atlanta, GA, United States*
F. Xavier Ruiz, Ph.D., *Center for Advanced Biotechnology and Medicine; Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey, United States*
Patrick R. Griffin, *The Scripps Research Institute, Jupiter, FL, United States*
Eddy Arnold, Ph.D., *Rutgers University - New Brunswick, Piscataway, New Jersey, United States*

The genome of SARS-CoV-2, the causative agent of the COVID-19 pandemic, is translated into two large polyproteins subsequently cleaved by viral papain-like protease and main protease (Mpro). We studied nsp7-11 polyprotein processing by Mpro and identified the cleavage order. We elucidated the integrative structures of the nsp7-11 polyprotein for the first time, unveiling the role of cleavage junction conformation and accessibility in determining the order of cleavage, with the nsp7-8 intermediate being cleaved last. Furthermore, this intermediate is incompletely processed even after 24 hours of exposure to Mpro. Its functional role in the viral cycle is unknown, but disruption of the nsp7-8 junction site in coronaviral reverse genetics systems is lethal, suggesting a unique drug target site. The precedent of bevirimat—a potent HIV maturation inhibitor that inhibits the cleavage event at the CA/SP1 junction of the Gag polyprotein—validates this concept. Additionally, hydrogen-deuterium exchange mass spectrometry (MS) and cross-linking MS of the Mpro:nsp7-11 complex revealed the footprint of the polyprotein binding on Mpro. Finally, a proteolysis assay using the nsp7-11 polyprotein as substrate—benchmarked with FDA-approved drug nirmatrelvir—was used to characterize the effect of inhibitors/binders on Mpro processing/inhibition. The results suggest that allosteric inhibition of Mpro may only be efficiently achieved by interface binders destabilizing the Mpro dimer. In summary, the structural insights into SARS-CoV-2 polyproteins may aid in understanding their role in the viral life cycle and provide a basis for their structure-based drug discovery.

019. Bemnifosbuvir (BEM, AT-527) a Potent Inhibitor of SARS-CoV-2 Variants of Concern (VOC), and a Promising Oral Antiviral with a High Resistance Barrier for Treatment of COVID-19 and Other Coronaviruses Infections

Qi Huang, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Steven Good, Ph.D., *Atea Pharmaceuticals, Inc., Massachusetts, United States*
Jonna B. Westover, Ph.D., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States*
Kie Hoon Jung, Ph.D., *Utah State University, Logan, Utah, United States*
Ashleigh Shannon, Ph.D., *Aix-Marseille Universite, Marseille, France*
Bruno Canard, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, , France*
Adel Moussa, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Jean-Pierre Sommadossi, Ph.D., *Atea Pharmaceuticals, Inc. emloyee, Boston, Massachusetts, United States*
Nancy Agrawal, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*

The continuing emergence of new SARS-CoV-2 variants is anticipated to lead to ongoing COVID-19 waves. New oral antivirals are urgently needed due to limitations of current antiviral options. BEM is an oral prodrug of a unique, 6-modified purine nucleotide which potently inhibits the polymerase of several single stranded RNA viruses including SARS-CoV-2 and other human coronaviruses. It is currently being evaluated in a Phase III study for the treatment of COVID 19. The antiviral activity of BEM was evaluated against SARS CoV-2 VOC in an in vitro model of normal human airway epithelial cells. BEM remains fully effective in vitro against all tested VOC, including 0.5-0.8 μM EC90s for Omicron variants. The in vitro activity of BEM against other human coronaviruses was also determined using cell lines able to metabolize BEM to its active triphosphate (BEM-TP). BEM exhibits antiviral activities against other human coronaviruses such as HCoV-229E, HCoV-OC43, and SARS-CoV in Huh7 cells with EC90 values of 1.2, 0.5, and 0.34 uM, respectively. An initial in vitro resistance study was conducted with the surrogate virus HCoV-229E in Huh7 cells. No polymerase mutations were identified to-date in or near the two BEM-TP binding sites (NiRAN domain and catalytic center) involved in replication inhibition. Two putative resistant variants (D773G, T780I in nsp12, not conserved in SARS-CoV-2) emerged at 9-10 passages with modest (3.7- to 5.4-fold) increases in EC50, and map to an α-helix, remote and distinct from both nucleotide binding sites. Given its potent activity against all coronaviruses tested, BEM is a promising therapeutic for future pandemic preparedness.

020. Combination of Antiviral Drugs Targeting SARS-CoV-2 RNA Polymerase and Exonuclease in vitro Demonstrates COVID-19 Therapeutic Potential

Carolina Sacramento, Ph.D., *Fiocruz, Rio de Janeiro, Brazil*
Xuanting Wang, *Columbia University, United States*
Steffen Jockusch, *Columbia University, United States*
Otavio Augusto Chaves, *Fiocruz, Brazil*
Natalia Fintelman-Rodrigues, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Rio de Janeiro, Brazil*
Jairo Ramos Temerozo, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Rio de Janeiro, Brazil*
Jingyue Ju, *Columbia University, United States*
Thiago Souza, *Fiocruz, Brazil*

SARS-CoV-2 variants continue to circulate due to the ability to escape the immune response induced by vaccines or prior infection. Although there are antivirals approved to treat COVID-19, such as the nucleoside analogues remdesivir and molnupiravir, clinical benefits are limited. The SARS-CoV-2 exonuclease-based proofreader removes nucleoside inhibitors incorporated into the viral RNA during replication, reducing the efficacy of these drugs. Combinations of inhibitors of viral RNA-dependent RNA polymerase (RdRp) and exonuclease (ExoN) could overcome this deficiency. We identified hepatitis C virus NS5A inhibitors pibrentasvir (PIB), ombitasvir (OMB) and daclatasvir (DCV) as SARS-CoV-2 ExoN inhibitors by molecular docking and enzymatic assays. In the presence of PIB, RNAs terminated with sofosbuvir (SFV), remdesivir (RDV), favipiravir (FVP), molnupiravir (MLP) and AT-527 were largely protected from excision by the exonuclease, while in its absence, there were rapid excised. Studies in Calu-3 cells demonstrated that SARS-CoV-2 susceptibility to clinically approved RdRp prodrugs - SFV, RDV, tenofovir and FVP, is enhanced by the ExoN inhibitors, PIB, OMB and DCV. These combinations strategy showed significant synergistic effects. Our data indicate that the use of RdRp and ExoN inhibitors as a combined therapy is expected to have enhanced efficacy in treating COVID-19. This combination approach may allow 2log10 reduction of virus replication in concentrations consistent with approved doses and plasma exposure. We anticipate that various nucleoside inhibitors of SARS-CoV-2 RdRp that are in clinical trials for COVID-19 will benefit from the addition of ExoN inhibitors.

021. EDP-235, an Oral 3CL Protease Inhibitor for the Treatment of COVID-19, Suppresses Viral Replication and Spread in SARS-CoV-2-Infected Ferrets

Michael Rhodin, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Bryan Goodwin, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Anand Balakrishnan, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Archie Reyes, *Enanta Pharmaceuticals, Watertown, MA, United States*
Michael Vaine, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Tessa Cressey, *Enanta Pharmaceuticals, Watertown, MA, United States*
Miranda Crepeau, *Enanta Pharmaceuticals, Watertown, MA, United States*
Nicole McAllister, *Enanta Pharmaceuticals, Watertown, MA, United States*
Ruichao Shen, *Enanta Pharmaceuticals, Watertown, MA, United States*
Kellye Daniels, *Enanta Pharmaceuticals, Watertown, MA, United States*
Daniel Leonard, *Enanta Pharmaceuticals, Watertown, MA, United States*
Indy Zang, *Enanta Pharmaceuticals, Watertown, MA, United States*
Lijuan Jiang, *Enanta Pharmaceuticals, Watertown, MA, United States*
Richard Plemper, *Georgia State University, Atlanta, GA, United States*
Guoqiang Wang, *United States*
Yat Sun Or, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*

EDP-235 is a potent and selective inhibitor of SARS-CoV-2 3C-like protease (3CLpro) under development for the treatment of COVID-19. In biochemical assays, EDP-235 inhibits 3CLpro from multiple SARS-CoV-2 lineages with half-maximal inhibitory concentrations (IC50) from 2.8¬–5.8 nM. In live virus assays against the SARS-CoV-2 Omicron strain

(B.1.1.529) in the presence of an efflux inhibitor, EDP-235 blocks virus-induced cytopathic effect with a 90%-maximal effective concentration (EC90) of 5.1 nM. The antiviral activity of EDP-235 was evaluated in a ferret model of acute SARS-CoV-2 infection and transmission. Therapeutic treatment of infected animals with EDP-235 (either 200 mg/kg once daily or twice daily) beginning 12 hours post-infection with SARS-CoV-2 (USA-WA1/2020) resulted in a rapid and sustained reduction in both live virus titer and viral RNA in nasal lavage samples. Only vehicle-treated animals had detectable live virus in their nasal turbinates 4 days post-infection demonstrating complete inhibition of viral replication by both EDP-235 dosing regimens. To evaluate the impact of EDP-235 treatment on SARS-CoV-2 transmission, infected source animals were co-housed with uninfected contact animals 60 hours post-infection. Live virus was recoverable from the nasal lavages of contact animals housed with vehicle-treated source animals from 12 hours onwards after co-housing. However, viral RNA and live virus were undetectable in nasal lavage and terminal nasal turbinate samples from contact ferrets co-housed with EDP-235-treated infected animals. Collectively, these data support the clinical evaluation of EDP-235 as an oral therapy for the treatment of COVID-19.

022. A Hinge Glycan Regulated Spike Bending Impacts Coronavirus Infectivity

Jing Jin, Ph.D., *Vitalant Research Institute, San Francisco, CA, United States*
Wah Chiu, Ph.D., *Stanford University, CA, United States*
David Chmielewski, Ph.D., *Stanford University, Palo Alto, CA, United States*
Muyuan Chen, Ph.D., *Stanford University, Palo Alto, CA, United States*
Greg Pintilie, Ph.D., *Stanford University, Palo Alto, CA, United States*
Eric Wilson, Ph.D., *Arizona State University, Tempe, AZ, United States*
Abhishek Singharoy, Ph.D., *Arizona State University, Tempe, AZ, United States*
Peng Zhao, Ph.D., *University of Georgia, Atlanta, GA, United States*
Lance Wells, Ph.D., *University of Georgia, Atlanta, GA, United States*
Graham Simmons, Ph.D., *Vitalant Research Institute, San Francisco, CA, United States*

Coronavirus spike (S) glycoproteins presented on the virion surface mediate receptor binding and membrane fusion during virus entry and constitute the primary target for vaccine and drug development. How the dynamics of the full-length spikes incorporated in viral lipid envelope correlates with the virus infectivity remains poorly understood. Here we present structures and distributions of native spike conformations on vitrified human coronavirus NL63 (HCoV-NL63) virions without chemical fixation by cryogenic electron tomography (cryoET) and subtomogram averaging along with site-specific glycan composition and occupancy determined by mass spectroscopy. Integration of native spike conformational dynamics with all-atom molecular dynamic simulations revealed a validated conformational landscape of the glycosylated, full-length spike and identified a novel role of stalk glycans in modulating spike bending. We showed glycosylation at N1242 alone at the upper portion of the stalk is responsible for the incredible orientational freedom of the spike crown. Functional assays suggest this glycan-dependent motion promotes virus infection. Our approach of integrating the conformational landscape determined by in situ cryoET, mass spectroscopy and in silico atomistic simulation can be valuable in assessing targets on various viral glycoproteins for the next generation of therapeutics and vaccines.

023. Development of Small Molecule Entry Inhibitors of Influenza A Viruses

Jazmin Galvan Achi, Ph.D. Seeking, *University of Illinois Chicago, Chicago, Illinois, United States*
Laura Cooper, Ph.D., *Illinois, United States*
Saad Alqarni, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States*
Ryan Bott, B.S., *University of Illinois Chicago, Chicago, Illinois, United States*
Norton P. Peet, Ph.D., *Chicago BioSolutions Inc., Chicago, Illinois, United States*
Irina N. Gaisina, Ph.D., *University of Illinois Chicago, Chicago BioSolutions Inc., Chicago, Illinois, United States*
Terry W. Moore, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States*
Lijun Rong, Ph.D., *University of Illinois Chicago, Chicago BioSolutions Inc., Chicago, Illinois, United States*

Influenza A viruses (IAVs) are a major public health concern as they are responsible for seasonal and pandemic influenza. IAVs are exceedingly transmissible and have a high morbidity and mortality rate, especially among high-risk populations. Their high mutation rate, due to antigenic shift and drift, leads these viruses to be increasingly difficult to treat with antiviral therapeutics, possibly leading to the emergence of pandemics. In response to these concerns, there is a need to continuously search for new antiviral drugs that can target drug-resistant IAV strains. From a high-throughput screen (HTS) we identified three hits, CBS1116, CBS117, and CBS1194. The first two are specific against

group 1 IAVs, while CBS1194 is specific against group 2 IAVs. From hit CBS1194, we developed a novel derivative, compound 4L. Using pseudotyped and infectious IAVs, we found that 4L displays nanomolar activity against group 2 IAVs. Molecular docking and HA mutagenesis studies determined that 4L interacts with the HA stalk region. In addition, from hits CBS1116 and CBS1117, we developed a novel derivative compound: ING-1466. Hit CBS1117 showed significant antiviral activity against group 1 IAVs, a co-crystal structure determined that it binds to the stalk region of HA5. Using this structure, ING-1466 was rationally designed. Our findings show that ING-1466 shows in vivo potency when administered orally and is able to inhibit group 1 IAVs alone or in combination with oseltamivir. Altogether, these novel derivatives exhibit potent activity against both group 1 and 2 IAVs by inhibiting viral entry, a promising development in the advancement toward new anti-influenza therapeutics.

024. Intermittent Therapy with IM 250, a Helicase Primase Inhibitor, Has Persistent Effects and May Reduce the Pool of Latent Reactivable Herpes Simplex Virus

Gerald Kleymann, Ph.D., *Innovative Molecules GmbH, Munich, Bavaria, Germany*
Nancy M. Sawtell, Ph.D., *University of Cincinnati, Ohio, United States*
Fernando J. Bravo, M.D., *Cincinnati Children's Hospital Medical Center CCHMC, Cincinnati, Ohio, United States*
David Dixon, M.S., *Cincinnati Children's Hospital Medical Center CCHMC, Cincinnati, Ohio, United States*
Christian Gege, Ph.D., *Innovative Molecules GmbH, Munich, Bavaria, Germany*
David I. Bernstein, M.D., *Cincinnati Children's Hospital Medical Center CCHMC, Cincinnati, Ohio, United States*

Background: Infection with herpes simplex viruses (HSV) type 1 and 2 are common but there are limitations to current antiviral therapies. Importantly, none of these therapies effect the pool of latent virus.

Methods: In mice we used HSV-1 17VP16pLz in the ocular infection model and heat stress to induce reactivation in vivo. In guinea pigs we used HSV-2 in the vaginal infection model where animals develop spontaneous recurrences. After latency was established, mice were randomized to receive either IM-250 or placebo treatment, while guinea pigs received either acyclovir, IM-250 or no treatment. ACV was provided in drinking water while IM-250 was supplied in the appropriate chow. Therapies were provided intermittently on schedules appropriate for each latency/reactivation model.

Results: In mice in two independent experiments 4 cycles of intermittent treatment with IM-250 starting 45 dpi significantly reduced the number of neurons undergoing reactivation in vivo compared to no therapy. In guinea pigs over a 6 month period intermittent therapy with IM-250 decreased recurrences compared to ACV. Reductions were seen both during the weeks of therapy as well during the no treatment weeks. Therapy with IM-250 also completely eliminated recurrences after 7 cycles of therapy while both other groups continued to develop recurrent disease. Following sacrifice, the number of explant reactivation events were reduced in the IM-250 group. Thus, there was evidence in both models that reactivation competent neurons were reduced.

Conclusion: IM-250 has distinct advantages over current antiviral HSV therapies and may decrease the pool of reactivatable virus, an unprecedented finding.

025. Intranasal Delivery of Fusion Inhibitory Lipopeptides Blocks SARS-CoV-2-induced Pathology In Mice and Permits Establishment of Long-lasting Protective Immunity

Branka Horvat, M.D., Ph.D., *International Center for Infectiology Research (CIRI), Lyon, France*
Valerie Favede, M.S., *International Center for Infectiology Research (CIRI), Département du Rhône, France*
Said Mougari, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, France*
Olivier Reyanrd, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, France*
Stephanie Durand, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, Rhone, France*
Magalie Mazelier, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, France*
Didier Decimo, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, France*
Nicole A. Lieberman, Ph.D., *University of Washington Medical Center, Seattle, United States*
Cyrille Mathieu, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, Rhone, France*
Guillaume Noel, Ph.D., *Institut Claude Bourgelat, Marcy l'Etoile, France*
Ana Zarubica, Ph.D., *Centre d'Immunophénomique, Aix Marseille Université, Inserm US12, CNRS, UAR3367, Marseille, France*
Camilla Predella, *Department of Pediatrics, Columbia University Medical Center, NY, United States*

Alexander L. Greninger, Ph.D., *University of Washington Medical Center, Seattle, WA, United States*
Christopher A. Alabi, Ph.D., *Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, United States*
Nicolino V. Dorrello, *Department of Pediatrics, Columbia University Medical Center, NY, United States*
Stephane Marot, Ph.D., *Sorbonne Université, Virology department, Pitié-Salpêtrière Hospital, AP-HP, Pierre Louis Epidemiology and Public Health institute (iPLESP), INSERM 1136, Paris, France*
Anne-Geneviève Marcelin, M.D., Ph.D., *Sorbonne Université, Virology Department, Pitié-Salpêtrière Hospital, AP-HP, Pierre Louis Epidemiology and Public Health institute (iPLESP), INSERM 1136, Paris, France*
Bernard Malissen, Ph.D., *Centre d'Immunophénomique, Aix Marseille Université, Inserm US12, CNRS, UAR3367, Marseille, France*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection continues to present a serious global health threat. We analyzed the capacity of fusion inhibitory peptides – derived from the heptad repeat domains in C-amino terminus HRC domain of the spike glycoprotein coupled to cholesterol – to inhibit SARS-CoV-2 infection in a murine model. Lipopeptides blocked SARS-CoV-2 infection both in cell lines and in organotypic cultures prepared from lungs of K18 mice expressing human ACE2 (K18-hACE2). Intranasal administration of peptides (4 mg/kg, given 3 times) reduced body weight loss, diminished virus shedding, and protected K18-hACE2 mice from the lethality induced by SARS-CoV-2 variants. The mice showed a decrease in viral load and normalization of the transcriptomic profile in lungs, including the expression of both innate and adaptive immunity gene clusters. Multiple administrations of high-dose lipopeptides had no adverse effects and did not interfere with peptide-induced antiviral protection during subsequent SARS-CoV-2 challenge. Finally, peptide-protected mice produced virus-neutralizing antibodies and were completely resistant to a second challenge with a lethal dose of SARS-CoV-2. These results suggest that immunization against future SARS-CoV-2 infection can occur even in the face of the preventive action of fusion inhibitory peptides. This strategy may offer a novel complementary approach to antiviral prophylaxis as part of the global effort against the current SARS-CoV-2 pandemic.

026. Ebola Virus Disease: In Vivo Protection Provided by the PAMP Restricted TLR3 Agonist Rintatolimod and Its Mechanism of Action

Angela Corona, Ph.D., *Molecular Virology Unit Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*
David R. Strayer, M.D., *AIM ImmunoTech, FL, United States*
Simona Distinto, Ph.D., *CADD unit Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*
Gian Luca Daino, Ph.D., *Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*
Annalaura Paulis, M.S., *Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*
Enzo Tramontano, Ph.D., *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
William M. Mitchell, M.D., Ph.D., *Vanderbilt University School of Medicine, C-3321 MCN Dept of Pathology, Microbiology and Immunology, Nashville, TN, United States*

Ebola virus (EBOV) is a highly infectious and lethal pathogen responsible for sporadic self-limiting clusters of Ebola virus disease (EVD) in Central Africa capable of reaching epidemic status. 100% protection from lethal EBOV-Zaire in Balb/c mice was achieved by rintatolimod (Ampligen) at the well tolerated human clinical dose of 6 mg/kg. The data indicate that the mechanism of action is rintatolimod's dual ability to act as both a competitive decoy for the IID domain of VP35 blocking viral dsRNA sequestration and as a pathogen-associated molecular pattern (PAMP) restricted agonist for direct TLR3 activation but lacking RIG-1-like cytosolic helicase agonist properties. These data show promise for rintatolimod as a prophylactic therapy against human Ebola outbreaks.

027. Drug Deconstructing and Re-engineering as an Alternative to Drug Repurposing

Consuelo B. Correa-Sierra, M.D., Ph.D., *Cornell University, Ithaca, NY, United States*
Chloe Monet-Murrell, Ph.D. Seeking, *Cornell University, NY, United States*
Mohamad Estaitie, Ph.D., *University of Alberta, Edmonton, AB, Canada*
Dennis Hall, Ph.D., *University of Alberta, Edmonton, AB, Canada*
LuisM Schang, D.V.M., Ph.D., *Cornell University, Ithaca, NY, United States*

Drug repurposing has been proposed as a shortcut towards antivirals. However, the many unsuccessful attempts to repurpose drugs against SARS-CoV-2 highlight the limitations of the concept, including PK/PD, the low probability of drugs highly optimized against one target having pharmacologically relevant activities against others, and their pharmacological effects precluding safe use in other diseases. Yet, drug repurposing makes use of the knowledge and investment in already developed drugs. We have explored an alternative to it, deconstructing and re-engineering existing drugs based on smaller simpler scaffolds. To this end, we screened in culture a limited number of truncated derivatives of mefloquine against human coronavirus OC43 (HCoV- OC43) using a CPE-based screen. Two scored as positive and seven as weakly positive. The two hits had unique commonalities, including absence of trifluoromethyl groups and presence of aromatic and alkylbenzene substituents. Both compounds were active against HCoV-OC43 and SARS-CoV-2 in viral replication assays but with limited potency and low selectivity. An initial exploration of the chemical space identified a lead scaffold that produced compounds preserving the potency while decreasing toxicity. The best ones have single digit micromolar potency and selectivity indexes larger than 10 to 20 (the highest concentration tested, 100 μM, did not result in 50% cytotoxicity). These compounds are active against two distantly-related coronaviruses and provide a scaffold for development based on a clinical drug. We are testing their activities against other viruses and identifying their mode of action to identify leads for further development.

028. Tracking the Journey Towards the Discovery of Raltegravir and Grazoprevir: Two Intriguing Tales On Antiviral Drug Discovery

Vincenzo Summa, Ph.D., *Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, Naples, Italy*

HIV and HCV differ significantly at the molecular level and with respect to their resultant pathogenesis. However, common features such as substantial genetic diversity, rapid turnover and high viral load together with the propensity to select for resistant variants and the need for chronic treatment (years and months for HIV and HCV, respectively) present similar challenges for the development of effective therapies. The presentation is a journey in the discovery and development process of two drugs, Raltegravir (IsentressTM), the first in class HIV integrase inhibitor approved, and Grazoprevir, a pan-genotype HCV protease inhibitor approved in combination with Elbasvir (ZepatierTM) to cure HCV patients in interferon free therapy. The key approaches, strategies from hit identification to PoC in animal model, candidate selection and clinical development including failure and success, will be described for both drugs focusing on the most important aspects of each process. Regarding HIV integrase, particularly relevant was the understanding of the hit mechanism of action and the required pharmacophore leading to the switch from HCV polymerase to HIV integrase, that combined with creative medicinal chemistry, led to the generation of a novel class of selective HIV integrase inhibitors allowing the selection and approval of Raltegravir. Instead, the profound analyses of the structural features of the different genotypes and mutants of the HCV protease, combining X-Ray and modelling studies, were the key elements for the discovery of Grazoprevir.

029. Perspectives for the Management of Cytomegalovirus Infections in the New Era of Antiviral Agents.

Jocelyne Piret, Ph.D., *CHU de Quebec-Laval University, Quebec City, Quebec, Canada*
Guy Boivin, M.D., *CHU de Quebec-Laval University, Quebec, Canada*

Cytomegalovirus (CMV) infections are responsible for serious complications in immunocompromised patients leading to increased morbidity and mortality. During decades, standard antiviral drugs to prevent or treat CMV infections and diseases were limited to (val)ganciclovir, foscarnet and cidofovir that all target the viral DNA polymerase. However, their administration can result in serious side effects and in the emergence of cross-resistance. In 2017, letermovir was approved for the prophylaxis of CMV infection in adult CMV-seropositive recipients of an allogeneic hematopoietic stem cell transplant. Four years later, maribavir was approved for the treatment of adult and pediatric transplant patients with CMV infection and disease refractory or resistant to DNA polymerase inhibitors. Letermovir targets the CMV terminase complex (formed by UL56, UL89 and UL51 subunits) and prevents the cleavage and encapsidation of viral DNA. Maribavir is an inhibitor of the UL97 kinase activity and interferes with capsid assembly and nuclear egress of virions. Thanks to their novel mechanisms of action, both drugs are effective against CMV isolates that are resistant to standard drugs and demonstrate more favorable safety profiles. In the clinic, mutations with a very high level of resistance to letermovir seem to emerge more rapidly in the UL56 gene than low-grade resistance mutations. Depending on their location in the UL97 kinase, mutations arising under maribavir can confer cross-resistance to ganciclovir. Further investigations are required to define the respective niche of letermovir and maribavir in the arsenal of drugs available for the management of CMV infections and diseases.

030. HIV Keeps on Surprising Us: A CRISPR-Cas Cure Adventure and a Drug-resistance Story

Ben Berkhout, Ph.D., *University of Amsterdam, Amsterdam, Netherlands*

The two hallmarks of the replication cycle of a retrovirus like the human immunodeficiency virus (HIV) are reverse transcription of the RNA genome into DNA and subsequent integration into the cellular genome. This integrated genome will persist for the life of the infected cell, which explains why it has not been possible to develop a cure, despite the availability of many potent antiviral drugs. To realize a cure, we developed CRISPR-Cas-based strategies to inactivate the integrated HIV genomes. We observed potent virus inhibition, but also surprising virus escape routes. A combination attack was designed that does not allow virus escape. But where is the virus hiding and how to reach those reservoirs? Inhibitors of the HIV integrase enzyme form an important drug class and Dolutegravir (DTG) is the WHO-preferred drug. Whereas resistance usually develops in the targeted HIV enzyme, in this case the Integrase, there is accumulating evidence for an unorthodox resistance mechanism that involved a regulatory HIV sequence, the polypurine track (PPT) that produces the primer for +strand synthesis during reverse transcription. It turns out that this PPT-mutated virus replicates - albeit at a slow pace – without integrating its DNA into the host cell genome! Details of this mechanism and several consequences will be discussed. In fact, this integration-minus HIV variant starts behaving like a non-integrating hepatitis B virus (HBV).

031. Combining Autofluorescent ANCHORTM Tagged Viruses with High Content Imaging for the Discovery of New Broad-spectrum Herpes Virus Inhibitors

Franck Gallardo, Ph.D., *NeoVirTech SAS, Toulouse, France*
Luigi Agrofoglio, Ph.D., *ICOA, France*
Vincent Roy, Ph.D., *ICAO, Orléans, France*
Elie Marcheteau, Ph.D., *NeoVirTech SAS, Toulouse, France*
Sandrine Kappler Gratias, Ph.D., *NeoVirTech SAS, Toulouse, France*
Charlotte Quentin-Froignant, Ph.D., *NeoVirTech SAS, Toulouse, France*
Megan Gribble Lloyd, Ph.D., *Upstate Medical University, Syracuse, New-York, United States*
Chloé Jacquet, Ph.D., *Université de Limoges, UMR 1092, Limoges, France*
Estelle Mosca, M.S., *Virology Unit, Microbiology and Infectious Diseases Department, Institut de Recherche Biomédicale des Armées, Brétigny-sur-Orge, France*
Frédéric Iséni, Ph.D., *Virology Unit, Microbiology and Infectious Diseases Department, Institut de Recherche Biomédicale des Armées, Brétigny-sur-Orge, France*
Sophie Alain, M.D., Ph.D., *Université de Limoges, UMR 1092, Limoges, France*
Jennifer Moffat, Ph.D., *Upstate Medical University, Syracuse, New-York, United States*

Herpesviruses are large dsDNA viruses, including Herpes simplex viruses, Cytomegalovirus and Varicella Zoster Virus that infect people worldwide. Although primary infection is often silent, herpesviruses remain in a latent form that can reactivate to trigger potentially life-threatening recurrences. Several antivirals have been developed, including DNA polymerase inhibitors (acyclovir, ganciclovir), terminase inhibitors (Letermovir), and primase inhibitors (Pritelivir). As repeated treatment with an antiviral can select for resistant strains, causing therapeutic failure, there is a constant need for the development of new antiviral therapies. To reach this goal, we developed a collection of autofluorescent ANCHOR tagged viruses, combined with high content imaging techniques, allowing the visualization of virus infection, replication, and propagation in living cells in the presence of a compound of interest. We also developed and optimized a collection of acyclonucleoside phosphonate molecules that inhibit Herpes viruses' replication in vitro. We selected LAVR-289 as a lead compound. LAVR-289 completely abolished viral replication at nM concentrations, which is at least 50 times more potent than other nucleoside inhibitors, and it was active against strains resistant to approved antiviral drugs. LAVR-289 prevented HCMV replication in human placenta villi ex vivo and VZV replication in a humanized mouse model. Interestingly, LAVR-289 inhibited the replication of all dsDNA virus tested so far, including the Mpox strain circulating in 2022 with an EC50 <100nM, by targeting a specific domain in the viral polymerase.

032. High Throughput Discovery of Small Molecular Inhibitors of Hepatitis B Virus Subviral Particle Biogenesis

Biplav Shrestha, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Sisi Yang, Ph.D., *China*
Lauren Griffith, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Julia Ma, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Fuxuan Wang, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Qiong Zhao, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Jimin Zhang, *Fudan University, China*
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Ju-Tao Guo, M.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

High levels of hepatitis B virus (HBV) surface antigen (HBsAg) in the blood of chronic carriers is considered to drive the exhaustion of antigen-specific T and B cells for the persistence of infection. Accordingly, therapeutic reduction, ideally, elimination of HBsAg should facilitate the recovery of host adaptive antiviral immune responses and functional cure of chronic hepatitis B. Recently, we discovered that an amphipathic alpha helix at the C-terminal region of antigenic loop of small envelop (S) protein plays an essential role in S protein oligomerization and morphogenesis of HBV subviral particles and disruption of the structure of alpha helix results in the degradation of S protein by 20S proteasomes (Sisi Yang, et al. J. Virol. 2021). We thus hypothesized that pharmacological disruption of subviral particle morphogenesis via specific targeting S protein oligomerization and subviral particle budding will reduce HBsAg. To discover small molecules with such an antiviral property, we established a cell-based assay for high throughput screen of compound library and assays for selection of screening "hits" that specifically inhibit subviral particle production. Our pilot screen campaign with a bioactive compound library identified the inhibitors of p97, MDM2 and 26S proteasomes significantly reduced the intracellular production of subviral particles and the function of those cellular proteins in HBV subviral particle biogenesis had been further validated by siRNA knockdown of their expression. In addition, 23 compounds that efficiently inhibit SVP production via distinct mechanisms will also be presented.

033. Cytosine Base Editing Inactivates the Hepatitis B Virus Episomal Genomic Reservoir and Integrated DNA

Anuj Kumar, Ph.D., *Cancer Research Center of Lyon, INSERM, Lyon, France*
Elena Smekalova, *Beam Therapeutics, Massachusetts, United States*
Maria Martinez, *Cancer Research Center of Lyon, INSERM, Lyon, France*
Emmanuel Combe, *Cancer Research Center of Lyon, INSERM, Lyon, France*
Anuj Kumar, *Cancer Research Center of Lyon, INSERM, Lyon, France*
Selam Dejene, *Beam Therapeutics, Cambridge, Massachusetts, United States*
Michael Packer, *Beam Therapeutics, Cambridge, Massachusetts, United States*
Dominique LeBoeuf, *Beam Therapeutics, Cambridge, Massachusetts, United States*
Luis Barrera, *Beam Therapeutics, Cambridge, Massachusetts, France*
Rosie Chen, *Beam Therapeutics, Cambridge, Massachusetts, United States*
Giuseppe Ciaramella, *Beam Therapeutics, Cambridge, Massachusetts, United States*
Barbara Testoni, *Cancer Research Center of Lyon, INSERM, Lyon, France*
Francine Gregoire, *Beam Therapeutics, Cambridge, Massachusetts, United States*
Fabien Zoulim, *Cancer Research Center of Lyon, INSERM; Hospices Civils de Lyon (HCL), France; University of Lyon, UMR_S1052, UCBL, Lyon, France; Institut Universitaire de France (IUF), Paris, France, Lyon, France*

There is an unmet need for HBV therapeutics that prevent rebound from viral genomic reservoir namely the covalently closed circular DNA (cccDNA), and silence the expression of HBsAg, produced in part from the integrated HBV DNA. This study aims to target HBV cccDNA and integrated viral DNA using Cytosine Base Editors (CBEs). A base editing strategy was devised to introduce stop codons in HBV genes, HBs and Precore, using two distinct gRNAs, named as gS and gPC, respectively. Transfection of HepG2-NTCP cells and primary human hepatocytes (PHHs) with CBE mRNA and gS+gPC combination enabled robust cccDNA editing leading to a sustained inhibition of HBsAg, HBeAg, 3.5kb viral RNA, and total intracellular HBV DNA. Importantly, gS+gPC prevented viral rebound compared to nucleoside analogue monotherapy in infected PHHs. Further, we observed a remarkable decline of HBsAg with HBs targeting gS (or gS+gPC) in HepG2.2.15 and PLC/PCRF/5 cells which harbor integrated HBV DNA sequences. The gS+gPC was

also assessed for its antiviral efficacy in the HBV minicircle mouse model. This in vivo model supports HBV replication and antigen expression resulting from hydrodynamic injection with a cccDNA-like plasmid. Hepatocellular delivery of the base editing reagents in this model was achieved via systemic administration of lipid nanoparticles (LNP) formulated with CBE mRNA and gS+gPC. Intravenous injection of this LNP formulation led to a significant reduction of HBV DNA, HBsAg and HBeAg in mouse serum. Taken together, the data suggest that base editing of HBV cccDNA and integrated DNA potently abrogate viral replication and HBs expression.

034. Optimization and Validation of a Rat HEV Transmission Model for Pre-clinical Evaluation of Novel Antiviral Molecules

Xin Zhang, M.S., *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Niels Cremers, *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium, Belgium*
Stijn Hendrickx, *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium, Belgium*
Lotte Coelmont, Ph.D., *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium, Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Suzanne Kaptein, Ph.D., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

Hepatitis E virus (HEV) causes acute or chronic viral hepatitis in humans. It is faeco-orally transmitted through contaminated drinking water or undercooked meat. While HEV variants causing human infection predominantly belong to the Orthohepevirus species A (HEV-A), an increasing number of human infections are caused by the rat HEV that belongs to the Orthohepevirus C species (HEV-C1). Thus, HEV-C1 should be considered an emerging cause of viral hepatitis in humans.Previously, we reported the high susceptibility of athymic nude rats to rat HEV infection when injected intravenously (i.v.) with infectious rat liver homogenate (PMID:27483350). Here, we explored the dynamics of HEV transmission in rats, and further characterized the rat HEV transmission model. Infectious HEV was found in the faeces of i.v. infected rats. We show that the transmission of rat HEV can occur via the faeco-oral route as faeces became positive after oral ingestion of infectious faeces. The transmission model was standardized by orally administrating the rats a faecal suspension containing pre-defined viral RNA copies. To validate the transmission model, the efficacy of ribavirin (RBV) against rat HEV was assessed. Faeco-orally inoculated rats treated with RBV (60 mg/kg for 12 days) were partially protected as they had significantly lower viral RNA levels (~2log10-fold less) in faeces and other tissues, compared to vehicle-treated rats.Altogether, we propose that the HEV-C1 transmission to humans occurs through exposure to infectious rat faeces. Our results also show that our athymic nude rat model is highly suitable for evaluating novel antiviral agents in blocking or delaying HEV transmission.

035. The Complex of NBD-14189 with HIV-1 Reverse Transcriptase and DNA Reveals Its Molecular Mechanism of Inhibition of Reverse Transcription

Natalie Losada, Ph.D. Seeking, *Center for Advanced Biotechnology and Medicine; Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey, United States*
F. Xavier Ruiz, Ph.D., *Center for Advanced Biotechnology and Medicine; Chemistry and Chemical Biology, Rutgers University, New Jersey, United States*
Francesca Curreli, Ph.D., *Lindsley F. Kimball Research Institute, New York Blood Center, New York City, New York, United States*
Kevin Gruber, *Center for Advanced Biotechnology and Medicine; Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey, United States*
Alyssa Pilch, *Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, New Jersey, United States*
Andrea Altieri, *EDASA Scientific, Scientific Park, Moscow State University, Moscow, Russia*
Alexander V. Kurkin, *EDASA Scientific, Scientific Park, Moscow State University, Moscow, Russia*
Kalyan Das, *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Asim K. Debnath, Ph.D., *Lindsley F. Kimball Research Institute, New York Blood Center, New York City, New York, United States*
Eddy Arnold, *Center for Advanced Biotechnology and Medicine; Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey, United States*

Human immunodeficiency virus (HIV) still classifies as an epidemic affecting 38.4 million people worldwide (WHO 2021). HIV-1 is usually treated with antiretroviral therapy comprising ≥2 drugs, including at least one HIV-1 reverse transcriptase (RT) inhibitor. FDA-approved drugs that target RT include nucleos(t)ide RT inhibitors (NRTIs) and nonnucleoside RT inhibitors (NNRTIs). NRTI-triphosphates inhibit elongation following binding at the polymerase active site and DNA incorporation. NNRTIs bind α10 Å away, in an allosteric pocket, from the polymerase active site. There is an imperative need for HIV-1 antivirals with new mechanisms of action that can evade drug resistance mutations in current strains. This study focuses on compounds ("NBD derivatives") originally developed to bind to HIV-1 gp120, some found to inhibit RT. Previously we determined structures of RT with NBD derivatives, observing binding in the primer grip region of the palm subdomain, bridging the dNTP and NNRTI-binding sites. The lead NBD derivatives have antiviral activity, low toxicity, and activity against RT polymerization (e.g., NBD-14189: EC50 = 89 nM, CC50 ≈ 21 μM, IC50 < 3 μM). Here we report insights into the mechanism of action of NBD derivatives against RT in presence of nucleic acid. The crystal structure of RT in complex with DNA and NBD-14189 reveals binding of the compound also occurs in the RT primer grip, which provokes a displacement of the 3' end of the DNA primer by ~3 Å away from the polymerase active site. This mechanism of action is reminiscent of the FDA-approved non-nucleoside RT inhibitors, albeit targeting a conserved pocket, anticipating a higher genetic barrier to resistance.

036. FXR Agonists Alone or In Combination with IFNa Inhibit HBV Replication and HDV Propagation In Functional Hepatocytes

Romain Barnault, Ph.D., *HepVir - CIRI - Inserm, Lyon, France*
Loranne Durimel, *HepVir - CIRI - Inserm, France*
Raphaël Darteil, Ph.D., *Enyopharma, Lyon, France*
Benoît De Chassey, Ph.D., *Enyopharma, Lyon, France*
Pietro Scalfaro, M.D., *Enyopharma, Lyon, France*
Jacky Vonderscher, Ph.D., *Enyopharma, Lyon, France*
David Durantel, Ph.D., *HepVir - CIRI - INSERM, Lyon, France*

The nuclear farnesoid X receptor (FXR) is a master regulator of hepatocyte differentiation and functions. Some agonists/ligands of FXR were shown to inhibit the replication of HBV and the propagation of HDV. Here, using seven different FXR agonists with different structures (GW4064, Tropifexor, Vonafexor, Cilofexor, Fexaramine and Nidufexor) we further characterized these inhibitory phenotypes in relevant in vitro models (PHH an dHepaRG), thus confirming a FXR-dependent class effect. IFNa (in its pegylated form) is yet often used as the first line treatment in HBV and HDV patients, despite its low tolerance and subsequent relative limited efficacy. The combination of Peg-IFNa with Vonafexor, a non-steroidal non-bile acid, and highly selective FXR agonist, has been shown, in an open-label phase II trial (NCT04365933), to significantly reduce HBsAg levels in HBe-negative HBV-infected patients. This stronger combination inhibitory phenotype has been recapitulated in our models with various FXR agonists against HBV and HDV replication in the absence of any drug toxicity. The inhibitory phenotype was particularly strong on HBV RNA and HBsAg biogenesis, as well as on the propagation of HDV by affecting the specific infectivity of secreted particles. FXR agonists show antiviral activity on both HBV and HDV viruses, with a much better efficacy when combined with low/less toxic doses of IFNa. This study provides support for the existence of a mechanism of action underlying the antiviral activity of FXR agonists, alone or in combination with IFNa, details of which should be explored further to assist on the identification of efficacy predictive factors for clinical evaluations

037. On Exploring the Structure-Activity Relationship of Nucleoside Phosphonates as Hepatitis B Virus (HBV) Inhibitors

Elisabetta Groaz, Ph.D., *Rega Institute, Medicinal Chemistry, Leuven, Belgium*

Small molecule oral treatments currently approved against hepatitis B virus (HBV) comprise nucleoside analogues [lamivudine, entecavir (ETV), and telbivudine] and acyclic nucleoside phosphonate prodrugs [adefovir dipivoxil, tenofovir disoproxil, and tenofovir alafenamide (TAF)]. However, these drugs are not effective for achieving HBV eradication, and chronic HBV infection remains endemic in many areas with an estimated 296 million people infected worldwide (2019, WHO) as well as the leading cause of end-stage liver diseases. Moreover, these figures can be expected to increase over the coming decade, particularly in low-income countries. Current efforts by our group are devoted to identifying novel nucleoside phosphonates that could inhibit the HBV polymerase to such an extent as to avoid recycling of nuclear covalently closed circular DNA (cccDNA) molecules that are responsible for viral persistence and reactivation. To this end, we synthesized three series of nucleoside phosphonate prodrugs featuring either a cyclic

(e.g., phosphonomethoxydeoxythreosyl) or acyclic [e.g., 3-fluoro-2-(phosphonomethoxy)propyl, 2-ethynyl-3-hydroxy-2-(phosphonomethoxy)propyl] sugar moiety connected to natural nucleobases, which exhibited potent in vitro anti-HBV activity. Effective synthetic routes were designed that allowed to gain access to multigram quantities of these compounds for further in vivo studies. Selected prodrugs were demonstrated to effectively inhibit the replication of ETV-resistant HBV and significantly reduce HBV cccDNA in cell-based assays. An antiviral efficacy comparable to TAF was observed for these prodrugs in a hydrodynamic injection-based HBV mouse model.

038. How a Love of RNA Biophysics Led to the Discovery of a Novel Antiviral Against Enteroviruses

Blanton S. Tolbert, Ph.D., *Case Western Reserve University, Cleveland, Ohio, United States*

Positive Strand RNA viruses persist to pose serious threats to human health and global economies. Disease progression mediated by viral pathogenesis requires numerous intersections between host proteins and viral RNA (vRNA) structures. Host-vRNA complexes drive essential processes in the replication cycles of viruses; as such, they represent untapped targets for therapeutic intervention. Members of the hnRNP family are cellular proteins frequently usurped by RNA viruses to modulate viral translation and genome synthesis. Moreover, recruitment of hnRNPs to viral RNA structures reprograms the cellular environment to favor optimal viral replication prior to triggering apoptosis. In my seminar, I will describe the mechanisms by which the mutually antagonistic hnRNP A1 and AUF1 proteins compete for the same vRNA structure to differentially regulate EV translation efficiency. Genetic mutations engineered to disrupt the structure of a conserved bulge of the SLII IRES domain inhibits viral replication by attenuating translation. By screening a library of small molecule RNA binders, we discovered that the compound DMA-135 binds SLII to dose-dependently inhibit viral replication by attenuating viral translation. Serial passaging of EV-A71 in the presence of low doses of DMA-135 selects for revertant viruses with suppressor mutations that map to the SLII bulge environment. Comparative structure-function studies reveal that the cellular mechanism of action of DMA-135 is to tip the SLII-hnRNP regulatory axis towards significantly lower levels of IRES-dependent translation, and the virus can compensate by evolving mutations that restore homeostasis. Our work defines the antiviral me

039. Understanding the Arenavirus-Host Cell Interface as a Guide to the Development of Novel Antiviral Approaches

Allison Groseth, Ph.D., *Friedrich-Loeffler-Institut, Greifswald - Insel Riems, Germany*

Despite being agents of severe human disease, therapeutic options for treating arenavirus infection remain extremely limited. Targeting host factors represents an attractive strategy; however, implementation is hindered by our lack of knowledge regarding virus-host interactions. Intriguingly, we have identified differences in the ability of highly pathogenic arenaviruses and their closely-related apathogenic relatives to regulate the induction of apoptosis during infection, and are thus interested in studying both the mechanism involved and its consequences for virus biology. In addition to identifying several proapoptotic factors responsible for triggering apoptosis in response to infection (i.e. p53, Puma, Noxa), we have recently demonstrated the upregulation of stress-activated protein kinases that appear to contribute to this process (i.e. p38, JNK). Activation of these kinases appears to be crucial for virus infection, while the late-stages of apoptotic cell death itself are not. Indeed, we have shown that the pathogenic Junín virus actively suppresses caspases activation by sacrificing a subset of its nucleoprotein as a decoy substrate for caspase cleavage, a process that may play further roles in the regulation of the antiviral response to infection. Given that apoptosis is a highly-druggable pathway that is already being exploited for cancer-therapeutics, we anticipate that a more nuanced understanding of the virus-host interface during apoptosis regulation may facilitate rational repurposing of available treatments in future.

040. Drug Repurposing at High Biocontainment: Lessons Learnt From Screening Against Ebola and SARS-CoV-2 Viruses

Robert A. Davey, Ph.D., *National Emerging Infectious Diseases Laboratories, Boston University, Boston, MA, United States*

Developing treatments for emerging infectious diseases is often hampered by obtaining sufficient financial support for screening large-scale small molecule libraries. While we and other groups have made advances, screening in a high biocontainment environment can also be slow. Each limitation often means the screening library's size is reduced to allow testing to occur in a reasonable time and cost. One approach to reduce screening library size is to evaluate libraries of existing drugs already being used in the clinic for treatment of other diseases. So called drug repurposing

was seen as having the potential to speed up drug development for emerging infectious diseases by targeting host pathways required for infection by pathogens. We have been involved in evaluating such libraries for development of treatments against Ebola virus and more recently, applied what we learnt to perform a screen for inhibitors of SARS-CoV-2 infection. Unfortunately, such screens involving highly active small molecules can create problems of how to prioritize hits for follow up. We will discuss our screening approach and lessons learnt for distinguishing useful hits from off-target outcomes by application of host cell protein networks based on virus replication dependencies. We will also describe our efforts to chemically retool hits toward useful lead compounds with heightened disease treatment efficacy.

041. Design of SARS-CoV-2 PLpro Inhibitors for COVID-19 Antiviral Therapy Leveraging Binding Cooperativity

Laura Cooper, Ph.D., *University of Illinois at Chicago, Chicago, IL, United States*
Zhengnan Shen, Ph.D., *University of Arizona, AZ, United States*
Kiira Ratia, Ph.D., *University of Illinois at Chicago, Chicago, IL, United States*
Gregory RJ Thatcher, Ph.D., *University of Arizona, Tuscon, AZ, United States*
Lijun Rong, Ph.D., *University of Illinois Chicago, Chicago BioSolutions Inc., Chicago, Illinois, United States*
Rui Xiong, Ph.D., *University of Arizona, Tuscon, AZ, United States*

The novel coronavirus (CoV) SARS-CoV-2 is the potentially deadly virus that led to the current coronavirus disease 2019 (COVID-19) pandemic. The SARS-CoV-2 mRNA vaccines have been effective at preventing severe morbidity and mortality. However, with the appearance of substantially mutated variants, it has become patently clear that vaccines alone will not suffice. Antiviral agents that complement vaccination are urgently needed to end the COVID-19 pandemic. The SARS-CoV-2 papain-like protease (PLpro), one of only two essential cysteine proteases that regulate viral replication, also dysregulates host immune sensing by binding and deubiquitination of host protein substrates. PLpro is a promising therapeutic target, albeit challenging owing to featureless P1 and P2 sites recognizing glycine. To overcome this challenge, we leveraged the cooperativity of multiple shallow binding sites on the PLpro surface, yielding novel 2-phenylthiophenes with nanomolar inhibitory potency. New co-crystal structures confirmed that ligand binding induces new interactions with PLpro: by closing the BL2 loop of PLpro forming a novel “BL2 groove” and mimicking the binding interaction of ubiquitin with Glu167 of PLpro. Together, this binding cooperativity translates to be one of the most potent PLpro inhibitors reported, with slow off-rates, improved binding affinities, and low micromolar antiviral potency in SARS-CoV-2-infected human cells.

042. Discovery of a Novel SARS-Cov2 Helicase Inhibitor from a 100K HTS Campaign

Donghoon Chung, Ph.D., *University of Louisville, Louisville, KY, United States*
Yuka Otsuka, Ph.D., *University of Florida, Scripps, Florida, United States*
Justin Shumate, Ph.D., *University of Florida, Scripps, Jupiter, Florida, United States*
HaJeung Park, Ph.D., *University of Florida, Scripps, Jupiter, Florida, United States*
Eunjung Kim, B.S., *University of Louisville, Louisville, Kentucky, United States*
Ozlem Demir, Ph.D., *University of California, San Diego, San Diego, California, United States*
Jie Li, Ph.D., *University of California, Berkeley, Berkeley, California, United States*
John Marecki, Ph.D., *University of Arkansas for Medical Science, Little Rock, Arkansas, United States*
Kevin Raney, Ph.D., *University of Arkansas for Medical Science, Little Rock, Arkansas, United States*
Teresa Head-Gordon, Ph.D., *University of California, Berkeley, Berkeley, California, United States*
Rommie Amaro, Ph.D., *University of California, San Diego, San Diego, California, United States*
Thomas Bannister, Ph.D., *University of Florida, Scripps, Jupiter, Florida, United States*
Louis Scampavia, Ph.D., *University of Florida, Scripps, Jupiter, Florida, United States*
Timothy Spicer, Ph.D., *University of Florida, Scripps, Jupiter, Florida, United States*

A novel therapeutic for SARS-CoV2 is urgently needed to treat the current and future pandemic. Viral RNA helicase is a critical component of the viral replicase complex and is essential for RNA virus replications. Viral RNA helicases can serve as a novel antiviral target for RNA viruses with a high barrier to drug resistance; however, viral helicases have not been studied well as an antiviral target. To discover novel SARS-CoV2 helicase inhibitors, an HTS-compatible enzymatic assay was developed that can measure the unwinding activity of double strand DNA by recombinant SARS-CoV2 helicase (nsP13) using a FRET-based readout. The assay was then implemented and optimized to 1536-well format with a robust performance. We conducted an HTS campaign with 100K small molecule compounds of Scripps

Drug Discovery Library and selected 521 primary hit compounds. Following dose-response and cheminformatics studies a total of 18 compounds emerged and were then tested for antiviral activity with live SARS-CoV2. A cell-based SARS-CoV2 assay identified a quinazoline compound as a hit compound with an EC50 of 3.83 and 0.49 μM in a CPE- and nLuc-based anti-SARS-CoV2 assays in cells. Using bioinformatical approaches, we identified a potential binding site of the hit compound near the 3′ RNA groove of SARS-CoV2 helicase model. This was further validated with a phenotypic resistance study using E142A mutant helicase, showing a decrease in sensitivity, suggesting the 3′ RNA binding groove as the potential action site. Overall our study shows that SARS-CoV2 helicase does serve as a novel antiviral target and our HTS approach can discover novel antiviral compounds targeting viral helicases.

043. The Viral Non-Structural Proteins as Antiviral Targets: From Screening to Hit Validation

Jean-Claude Guillemot, Ph.D., *AIX Marseille University, Marseille, France*
Cecilia Eydoux, Ph.D., *Aix Marseille University, France*
Mikael Feracci, Ph.D., *AFMB UMR 7257 CNRS, AMU, MARSEILLE, 13008, France, Metropolitan*
Lea Lo Bello, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Veronique Fattorini, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Barbara Selisko, Ph.D., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Claire Zimberger, *Marseille, France*
Ashleigh Shannon, Ph.D., *Aix-Marseille Universite' employee, Marseille, France*
Karine Alvarez, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France, Marseille, France*
Etienne DECROLY, Ph.D., *AFMB UMR 7257 CNRS, AMU, MARSEILLE, 13288, France*
François Ferron, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France, Marseille, France*
Bruno CANARD, Ph.D., *AFMB UMR 7257 CNRS, AMU, MARSEILLE, 13288, France*

Identification of new antivirals is determinant to control the dissemination of emerging viruses. Strategies based on targeting viral non-structural protein have shown to be successful in providing direct-acting antivirals with high specificity. During the ongoing COVID-19 pandemic, however, and despite an unseen international mobilization, it was unfortunately shown how difficult it is to identify new antiviral drugs. Almost all existing drugs have been evaluated, with modest success up to now. To reach the goal of identifying antivirals against emerging viruses, combination of knowledges based on fundamental research and screening strategies to a bigger scale have to be developed. We have developed screening assays against the Coronavirus and Flavivirus replication complexes, mainly based on the polymerase catalytic activity and/or Protein-Protein Interactions. The use of new chemical libraries, including the exploration of new chemical spaces, is ongoing with stimulating results. Enzyme-based screening assays efficiently complement large-scale screening campaign narrowed to focused libraries, and are a powerful guide for the hit validation and hit-to-lead process. We have developed several powerful target validation strategies using a panel of biophysics techniques (eg., Thermal Shift Assays) on a broad catalogue of essential non-structural viral proteins, hastening the entry of molecules in crystallography or cryo-microscopy programs.

044. Antiviral Activity of Viperin-Inspired 3′-Deoxy-3′,4′-didehydro-nucleoside Phosphoramidate Prodrugs

Samantha Kennelly, Ph.D. Seeking, *Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota, United States*
Jacob Sawyer, Ph.D. Seeking, *Department of Chemistry, University of Minnesota, Minnesota, United States*
Anne Payne, B.S., *The Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, Slingerlands, New York, United States*
Jamie Arnold, Ph.D., *University of North Carolina - Chapel Hill, Department of Microbiology and Immunology, Chapel Hill, NC, United States*
Craig Cameron, Ph.D., *Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, United States*
Alexander Ciota, Ph.D., *The Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, Department of Biomedical Sciences, State University of New York at Albany School of Public Health, Slingerlands, New York, United States*
Daniel Harki, Ph.D., *Department of Medicinal Chemistry and Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, United States*

There is an urgent need for the continued development of potent and safe broad-spectrum antiviral agents as evidenced by the outbreak of coronavirus disease 2019 and the emergence of additional viruses of pandemic concern, such as monkeypox in 2022. Nucleoside analogues have been a tremendously successful platform for developing novel therapeutic molecules for viral infections. In 2018, it was discovered that the antiviral enzyme viperin catalyzes the formation of a nucleotide analogue, 3′-deoxy-3′,4′-didehydro-cytidine-5′-triphosphate (ddhCTP). ddhCTP is incorporated into viral genomes and terminates genomic replication to confer antiviral effects. Strikingly, viperin activation results in broad-spectrum activity against both RNA and DNA viruses. Additionally, ddhCTP has no obvious toxicity to human cells. Unfortunately, ddhCTP is not cell permeable, which necessitates the development of a translationally viable molecule to enable the efficient production of ddhCTP in cells. One strategy to accomplish this goal is the utilization of phosphoramidate pronucleotides (ProTides). Here, we show that ddhC ProTides produce significant antiviral activity in both West Nile and Zika Virus infection models. In addition, we also show structure activity relationship (SAR) studies of related ddh-nucleoside ProTides and report their antiviral activity and favorable cytotoxicity profiles in effort to develop more potent antiviral agents. Collectively, this work advances the development of new antiviral agents for forthcoming viral pandemics.

045. JNJ-A07 Targets the Dengue Virus NS4A-2K-NS4B Interaction with NS3 and Blocks De Novo Formation of Vesicle Packets

Dominik Kiemel, Ph.D. Seeking, *Heidelberg University, Heidelberg, Baden-Württemberg, Germany*
Ann-Sophie Kröll, B.S., *Heidelberg University, Germany*
Solène Denolly, Ph.D., *Heidelberg University, Heidelberg, Germany*
Uta Haselmann, *Germany*
Jean-François Bonfanti, Ph.D., *Galapagos N.V., Romainville, France*
Jose Ignacio Andres, Ph.D., *Janssen R&D, Discovery Chemistry, Toledo, Spain*
Brahma Ghosh, Ph.D., *Janssen R&D, Discovery Chemistry, Spring House, PA, United States*
Marnix Van Loock, Ph.D., *Janssen Pharmaceutica NV, Beerse, Belgium*
Olivia Goethals, *Global Public Health R&D, Janssen Pharmaceutica NV, Beerse, Belgium*
Ralf Bartenschlager, Ph.D., *Heidelberg University, Heidelberg, Germany*

Despite an urgent global need, pharmacological interventions for the treatment or prophylaxis of dengue disease are not yet available. Recently, we described JNJ-A07, an NS4B inhibitor exhibiting potent antiviral activity against strains belonging to the four serotypes of dengue virus (DENV). Here, we report results of a detailed mechanism-of-action study. Using a photoaffinity derivative of JNJ-A07, we observed specific accumulation of the compound at intracellular structures enriched for DENV NS3 and NS4B. We elucidated that JNJ-A07 targets a previously unknown interaction between the NS2B-NS3 protease complex and the cleavage precursor NS4A-2K-NS4B and arrests the de novo formation of vesicle packets, the putative sites of viral RNA replication. JNJ-A07 resistance mutants retained both the interaction with the precursor and vesicle packet formation in the presence of the compound. NS4B mutants blocking the interaction between NS2B-NS3 and NS4A-2K-NS4B are unable to form vesicle packets, suggesting that both processes are functionally linked. In summary, we unravelled the antiviral mechanism of a novel class of DENV inhibitors, contributing important insights into the formation of the viral replication organelle and its critical role in the viral life cycle.

046. AT-752 Targets Multiple Sites and Activities on the Dengue Virus Replication Enzyme NS5

Mikael Feracci, Ph.D., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Cécilia Eydoux, *AFMB, CNRS, Aix-Marseille University, UMR 7257, France*
Veronique Fattorini, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Lea Lo Bello, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Pierre Gauffre, *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Barbara Selisko, Ph.D., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Priscila Sutto-Ortiz, *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Ashleigh Shannon, Ph.D., *Aix-Marseille Universite' employee, Marseille, France*
Hongjie Xia, *United States*
Pei-Yong Shi, *United States*

Mathieu Noel, *IBMM, UMR 5247 CNRS-UM1-UM2, Department of Nucleic Acids, Montpellier University, France*
Françoise Debart, *IBMM, UMR 5247 CNRS, Université de Montpellier, ENSCM, France*
Jean-Jacques Vasseur, *IBMM, UMR 5247 CNRS-UM1-UM2, Department of Nucleic Acids, Montpellier University, France*
Steven Good, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Kai Lin, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Jean-Pierre Sommadossi, Ph.D., *Atea Pharmaceuticals, Inc. employee, Boston, Massachusetts, United States*
Aurélie Chazot, *AFMB, CNRS, Aix-Marseille University, UMR 7257, France*
Karine Alvarez, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France, Marseille, France*
Jean-Claude Guillemot, *AFMB, CNRS, Aix-Marseille University, UMR 7257, France*
Etienne Decroly, Ph.D., *AFMB UMR 7257 CNRS, AMU, MARSEILLE, 13288, France*

AT-752 is a guanosine analogue prodrug active against dengue virus (DENV). In infected cells, it is metabolized into 2′-methyl-2′-fluoro guanosine 5′-triphosphate (AT-9010) which inhibits RNA synthesis in acting as a RNA chain terminator. Here we show that AT-9010 has several modes of action on DENV full-length NS5. AT-9010 does not inhibit the primer pppApG synthesis step significantly. However, AT-9010 targets two NS5-associated enzyme activities, the RNA 2′-O-MTase and the RNA-dependent RNA polymerase (RdRp) at its RNA elongation step. Crystal structure and RNA methyltransferase (MTase) activities of the DENV 2 MTase domain in complex with AT-9010 at 1.97 Å resolution shows the latter bound to the GTP/RNA-cap binding site, accounting for the observed inhibition of 2′-O but not N7-methylation activity. AT-9010 is discriminated ~10 to 14-fold against GTP at the NS5 active site of all four DENV1-4 NS5 RdRps, arguing for significant inhibition through viral RNA synthesis termination. In Huh7 cells, DENV1-4 are equally sensitive to AT-281, the free base of AT-752 (EC50 ≈ 0.50 μM), suggesting broad spectrum antiviral properties of AT-752 against flaviviruses.

047. Five Cellular Enzymes In the Activation Pathway of Bemnifosbuvir, a Drug-candidate Against SARS-CoV-2 Infections

Aurélie Chazot, M.S., *AFMB UMR 7257, Marseille, France*
Claire Zimberger, *France*
Mikael Feracci, Ph.D., *AFMB UMR 7257 CNRS, AMU, MARSEILLE, 13008, France, Metropolitan*
Sergio Hernandez, *France*
Camille Falcou, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, 13009, Marseille, France*
Adel Moussa, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Steven Good, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Jean-Pierre Sommadossi, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
François Ferron, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France, Marseille, France*
Karine Alvarez, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France, Marseille, France*
Bruno Canard, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, 13288, France*

The current pandemic of SARS-CoV-2 has caused substantial health issues and emphasizes the immediate need of powerful antivirals. Nucleosi/tide analogues have proved their efficiency as polymerase inhibitors against many viruses and hold a place of choice in the fight against coronaviruses. Bemnifosbuvir (AT-527), a phosphoramidate nucleotide analogue prodrug, recently entered phase III clinical trials for the treatment of COVID-19. Once in cells, AT-527 is converted into its triphosphate form, AT-9010, that targets the nsp12 gene product at both its viral RNA-dependent RNA polymerase and nucleotidyltransferase activity, accounting for its antiviral effect. Because the conversion of a prodrug into its active form is crucial for its final antiviral efficiency, we aim to provide a better understanding of the key enzymes dictating the metabolic pathway of AT-527. Here, we have identified five enzymes allowing the activation of AT-527 into its active principle AT-9010. These five enzymes reconstituting the metabolic pathway were expressed, purified, and shown to catalyze qualitatively and quantitatively reactions on their respective metabolites, in an ordered pathway. The specificity of some key enzymes together with crystallographic structures of enzyme/substrate co-complexes points to new possibilities to design improved nucleotide analogues as well as should help the understanding and use of the most relevant cellular, tissue, and animal models.

048. Treatment of Yellow Fever Virus with the NS4B Inhibitor BDAA and Effects on RNA-Sensing Innate Immune Pathways in a Hamster Model

Abbie E. Weight, B.S., *Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Shawn Zimmerman, D.V.M., Ph.D., *Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Utah, United States; Utah Veterinary Diagnostic Laboratory, Logan, UT, United States*
Arnaud van Wettere, *Utah Veterinary Diagnostic Laboratory, Logan, UT, United States*
Venkatraman Siddharthan, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Justin Julander, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, UT, United States*

Yellow fever virus (YFV) is a mosquito-borne flavivirus with considerable burden in South America and Africa and no approved antivirals. BDAA is an orally available YFV NS4B inhibitor with activity against YFV in cell culture and a hamster model. Along with directly inhibiting virus replication, treating YFV-infected cells with BDAA increases RNA-sensing pathway-mediated immune responses by releasing YFV replication intermediates into the cytoplasm. While previously demonstrated in cell culture, it is important to understand if this immune effect is relevant to BDAA's efficacy in vivo. We used qRT-PCR and TUNEL staining to demonstrate the effect of BDAA treatment of YFV on RNA-sensing pathway-related cytokines and apoptosis in liver tissue in a hamster model. We also reaffirm BDAA's in vivo efficacy against YFV. Administration of 200 mg/kg/d BDAA, initiated 4 hours pre-infection or 2 days post infection (dpi) and continuing for 7 days, significantly improved survival, serum viremia, weight change, and serum alanine aminotransferase (ALT) concentrations in YFV-infected hamsters. Treatment initiated 4 hours pre-infection and 2 dpi both resulted in 100% survival compared to total mortality in placebo-treated hamsters ($p<0.0001$ for both). BDAA treatment initiated 2 dpi resulted in a 2.5 log10-fold decrease in viremia compared with placebo controls ($p<0.0001$). Histopathology showed decreased necrosis in liver tissue when treatment is initiated 2 dpi compared to placebo-treated animals and no visible liver necrosis in prophylactic treatment. These results further demonstrate BDAA's vivo efficacy against YFV and support the potential of NS4B inhibitors as treatments for YFV.

049. New Class of Small Molecules that Inhibits Yellow Fever Virus by Targeting the NS4B Protein

Alina Soto, Ph.D. Seeking, *KU Leuven, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, Leuven, Belgium*
Rana Abdelnabi, Ph.D., *Rega Institute for Medical Research, Belgium*
Joyce Van Bree, *University of Wageningen, Wageningen, Netherlands*
Suzanne Kaptein, Ph.D., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Ahmed Mohamed Elsayed Mohamed, *Pirbright Institute, Surrey, United Kingdom*
Linda P. Salekwa, *Mbeya University of Science and Technology, Mbeya, Tanzania*
Irina V. Il'ina, *Novosibirsk Institute of Organic Chemistry, Russia*
Oksana S. Patrusheva, *Novosibirsk Institute of Organic Chemistry, Russia*
Dina V. Korchagina, *Novosibirsk Institute of Organic Chemistry, Russia*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Konstantin P. Volcho, *Novosibirsk Institute of Organic Chemistry, Russia*
Nariman F. Salakhutdinov, *Novosibirsk Institute of Organic Chemistry, Russia*
Leen Delang, Ph.D., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

The yellow fever virus (YFV) is a mosquito-borne virus causing acute haemorrhagic fever in humans. Because of its high morbidity, recurring outbreaks, vaccine shortages, and lack of antiviral drugs, the development of safe and effective inhibitors of this virus is needed. Here, we report on the identification of a new class of YFV inhibitors targeting the NS4B viral protein. From a series of heterocyclic compounds with hexahydro-2H-4,6-(epoxymethano)chromene moieties, compound I7-20-1 had the most potent antiviral effect in a cytopathogenic effect-reduction assay in Huh7 cells (EC50 = 2.1 ± 0.2 µM, CC50 = 60 µM). In a virus yield assay, I7-20-1 reduced the infectious virus titer with ~3 log10. No antiviral activity was observed against other flaviviruses nor enteroviruses, suggesting YFV-specific antiviral activity. Time-of-addition experiments showed that the antiviral effect of I7-20-1 decreased when added 4 hours post-infection, indicating that I7-20-1 acts early in the replication cycle. To elucidate the molecular target of I7-20-1, a resistant YFV variant was generated with a fold resistance of >25. A single mutation (I84T) was identified in the NS4B gene. This isoleucine is located within the transmembrane domain 2. Other YFV NS4B inhibitors (BDAA and CCG-4088) were

previously described, but resistance was reported in different regions of NS4B, suggesting another mechanism of action. Cross-resistance assays with these inhibitors are currently ongoing, as well as reverse-engineering of the YFV NS4B-I84T mutant. In summary, we identified a new class of YFV inhibitors with a resistant genotype different from that of previously described YFV NS4B inhibitors.

050. Molecular Architecture of the Chikungunya Virus Replication Complex for Antiviral Development

EMBO YOUNG INVESTIGATOR LECTURE

Dahai Luo, Ph.D., *Nanyang Technological University, Singapore, Singapore*
Yaw Bia Tan, Ph.D. Seeking, *Nanyang Technological University, Singapore, Singapore*
David Chmielewski, Ph.D., *Stanford University, Palo Alto, CA, United States*
Michelle Cheok Yien Law, *Nanyang Technological University, Singapore, Singapore*
Kuo Zhang, Ph.D., *Nanyang Technological University, Singapore, Singapore*
Yu He, *Nanyang Technological University, Singapore, Singapore*
Muyuan Chen, Ph.D., *Stanford University, Palo Alto, CA, United States*
Jing Jin, *United States*

To better understand how positive-strand (+) RNA viruses assemble membrane-associated replication complexes (RCs) to synthesize, process and transport viral RNA in virus-infected cells, we determined both the high-resolution structure of the core RNA replicase of chikungunya virus and the native RC architecture in its cellular context at subnanometer resolution, using in vitro reconstitution and in situ electron cryotomography, respectively. Within the core RNA replicase, the viral polymerase nsP4, which is in complex with nsP2 helicase-protease, sits in the central pore of the membrane-anchored nsP1 RNA-capping ring. The addition of a large cytoplasmic ring next to the C-terminus of nsP1 forms the holo-RNA-RC as observed at the neck of spherules formed in virus-infected cells. These results represent a major conceptual advance in elucidating the molecular mechanisms of RNA virus replication and the principles underlying the molecular architecture of RCs, likely to be shared with many pathogenic (+) RNA viruses. Overall, the CHIKV RC structure provides the molecular basis of viral RNA replication and serves as a useful tool for antiviral development against alphaviruses and other (+) RNA viruses.

051. The MEK1/2 Inhibitor Zapnometinib Is Safe and Well Tolerated In Humans and Has Both, Anti-SARS-CoV-2 As Well As Immunomodulatory Activity

Oliver Planz, Ph.D., *Eberhard Karls University, Tübingen, Germany*
Helen Hoffmann, Ph.D. Seeking, *Atriva Therapeutics GmbH, Tübingen, Germany*
Andre Schreiber, Ph.D., *Germany*
Julia Koch-Heier, Ph.D. Seeking, *Germany*
Hazem Hamza, Ph.D. Seeking, *Germany*
Lara M. Schüssele, M.S., *Germany*
Sebastian Canisius, M.D., *Germany*
Aileen Faist, Ph.D. Seeking, *Germany*
Linda Brunotte, Ph.D., *Germany*
Nigel Horscroft, Ph.D., *Germany*
Martin Bauer, M.D., *Atriva Therapeutics GmbH, Tübingen, Germany*
Stephan Stenglein, M.D., *Atriva Therapeutics GmbH, Germany*
Michael Schindler, Ph.D., *Germany*
Stephan Ludwig, Ph.D., *Germany*

In the disease course of severe COVID-19, the virus only represents the trigger in the early stage of infection while in later stages the disease rather depends on the hyper-induction of proinflammatory cytokines/chemokines. The cellular Raf/MEK/ERK signalling pathway is hijacked by many viruses like influenza virus (IV) or respiratory syncytial virus (RSV) to ensure their propagation. Since, the pathway is also known to be involved in the regulation of cytokine and chemokine responses, blockade of the signalling cascade appears to be a promising therapeutic strategy to target the full course of hyperinflammatory viral diseases. Here, we show that zapnometinib, an inhibitor of the host cell kinase MEK, meets these requirements for an anti-COVID-19 drug perfectly. The drug shows efficacy against SARS-CoV-1,

MERS and all tested variants of SARS-CoV-2 in vitro and acts antiviral in vivo in the Syrian hamster model. We also found a strong synergistic action with licenced direct-acting antivirals. Zapnometinib reduced cytokine/chemokine expression in particular for such that are involved in COVID-19 e.g. MCP-1, IP-10, TNF-α. In a double-blind, first-in-human phase 1 trial (NCT04385420) we found favourable pharmacokinetics for zapnometinib with single doses up to 900 mg and multiple doses up to 600 mg daily for seven days that were safe and well tolerated in healthy volunteers. The present data formed the basis for a phase 2 clinical trial of hospitalized COVID-19 patients (RESPIRE; NCT04776044). Since zapnometinib also shows efficacy against other RNA viruses such as IV, RSV or hantavirus, it could be considered as a broad-spectrum antiviral drug and for pandemic preparedness.

052. AI-driven Approach to the Discovery of Novel Mpro Inhibitors with High Pan-coronavirus Activity

Marco Derudas, Ph.D., *Exscientia, Oxford, United Kingdom*
Simon Richards, M.S., *Exscientia, United Kingdom*
Lucy Ellis, *Exscientia, Oxford, United Kingdom*
Nanna Ahlsten, *Exscientia, Oxford, United Kingdom*
Magd Badaoui, *Exscientia, Oxford, United Kingdom*
Konstantinos Papachristos, *Exscientia, Oxford, United Kingdom*
Giovanni Bocci, *Exscientia, Oxford, United Kingdom*
Alberto Bresciani, Ph.D., *Exscientia, Oxford, United Kingdom*
Fraser Cunningham, *Exscientia, Oxford, United Kingdom*
Chris Radoux, *Exscientia, Oxford, United Kingdom*
Simone Culurgioni, *Exscientia, Oxford, United Kingdom*
Iva Navratilova, *Exscientia, Oxford, United Kingdom*
Sean Robinson, *Exscientia, Oxford, United Kingdom*
Miriam Pasquini, *Exscientia, Oxford, United Kingdom*
Maria Serova, *Exscientia, Oxford, United Kingdom*
Philipp Schäfer, *Exscientia, Oxford, United Kingdom*
Louise Bird, *Exscientia, Oxford, United Kingdom*
Renata Filler, *Yale University, Departments of Laboratory Medicine and Immunobiology, New Haven, Connecticut, United States*
Jin Wei, *Yale University, Departments of Laboratory Medicine and Immunobiology, New Haven, Connecticut, United States*
Mia Madel Alfajaro, *Yale University, Departments of Laboratory Medicine and Immunobiology, New Haven, Connecticut, United States*

The COVID-19 pandemic, caused by SARS-CoV-2, demonstrated worldwide vulnerability to emerging viruses and highlighted the lack of availability of antivirals with an appropriate spectrum of activity. In this context, the research and development of oral antiviral therapeutics with broad-spectrum activity against novel SARS-CoV-2 variants, other human coronaviruses (i.e. MERS-CoV, SARS-CoV), and pre-emergent bat coronaviruses are still urgently needed. The SARS-CoV-2 main protease (Mpro), a key enzyme in viral replication, was identified as an attractive target for an artificial intelligence (AI) driven drug discovery effort by an internal tractability analysis. A key contributing factor to the selection of Mpro as a target was the high degree of sequence conservation with other known human coronaviruses, as we believed this would enable the development of compounds with high pan-coronavirus activity acting on known and potentially emerging new variants of the virus. Compound design was driven by generative design algorithms, which have the advantage of iteratively generating large libraries of compounds covering wide chemical and property space. These compounds were then scored and filtered against machine learning (ML) models to efficiently select the highest scoring (potency, selectivity and drug-like properties) molecules for synthesis. In this presentation, we will report on the AI and ML-driven design strategies and drug discovery activities leading to the identification of highly potent, broad spectrum Mpro inhibitors.

053. Enteric Viruses Replicate In Salivary Glands and Infect Through Saliva

Nihal Altan-Bonnet, Ph.D., *National Institutes of Health, USA, Bethesda, Maryland, United States*

Enteric viruses like norovirus, rotavirus and astrovirus have long been accepted as spreading in the population through fecal-oral transmission: viruses shed into feces from one host and enter the oral cavity of another, bypassing salivary glands and reaching the intestines to replicate, shed in feces and repeat the transmission cycle. Yet, there exist viruses (e.g., rabies) that infect the salivary glands, making the oral cavity one site of replication and saliva one conduit of transmission. Here I present data showing that enteric viruses productively and persistently infect salivary glands, reaching titers comparable to that in intestines. We demonstrate that enteric viruses get released into the saliva, identifying a second route of viral transmission. This is particularly significant for infected infants whose saliva directly transmits enteric viruses to their mothers' mammary glands through backflow during suckling. This sidesteps the conventional gut-mammary axis route and leads to a rapid surge in maternal milk IgA antibodies. Collectively our research uncovers a new transmission route for enteric viruses with implications for therapeutics, diagnostics, and importantly sanitation measures to prevent spread through saliva.

054. Computer-aided Approaches Towards the Development of Small-molecule Antivirals for Norovirus Infections

Marcella Bassetto, Ph.D. Seeking, *Swansea University, Swansea, Wales, United Kingdom*

Human norovirus represents the most common cause of acute gastroenteritis outbreaks worldwide. Infection with this virus can lead to severe complications in young children, especially in developing countries, in the elderly, and in immunocompromised patients. While no vaccines or antiviral options are currently approved for clinical use, the development of antiviral therapeutics is needed, to be used both as treatment options and as prophylactic measures during outbreaks. In our research group, we have used different computer-aided techniques to identify novel small molecules that interfere with norovirus replication. These methods were mainly applied to the study of the viral RNA-dependent RNA-polymerase (RdRp), to develop inhibitors of its essential function for the viral genome replication, while the potential inhibition of the viral non-structural protein 1/2 (NS1/2) was also explored. A combination of structure- and ligand-based methods led to the characterisation of promising hit candidates, able to inhibit the activity of the viral RdRp in biochemical assays. These biochemical hits were optimised into novel chemical entities able to prevent the virus from replicating in cell-based systems, and in a zebrafish animal model. The synthetic chemistry approaches applied to elucidate structure-activity relationships (SARs) and perform early hit-to-lead optimisation studies for these molecules will be presented. Preliminary results obtained from a docking-based virtual screening on a structural model for the viral NS1/2 protein will also be discussed.

055. Anti-hepatovirus Activity of TENT4A/B Inhibitors

You Li, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*
Ichiro Misumi, *University of North Carolina at Chapel Hill, NC, United States*
Yanming Du, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Stanley M. Lemon, *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Despite excellent vaccines, resurgent outbreaks of hepatitis A have caused thousands of hospitalizations and hundreds of deaths within the U.S. in recent years. There is no effective antiviral therapy, and many aspects of the hepatitis A virus (HAV) replication cycle remain to be elucidated. HAV replication requires the zinc-finger protein ZCCHC14 and noncanonical TENT4 poly(A) polymerases with which it associates, but the underlying mechanism is unknown. The ZCCHC14/TENT4 complex is also a host factor for hepatitis B virus (HBV), where it lengthens and stabilizes the 3′ poly(A) tails of viral mRNAs. However, chemical inhibition of TENT4A/B with the dihydroquinolizinones (DHQ) RG7834 had no impact on the length of the HAV 3′ poly(A) tail, stability of HAV RNA, or cap-independent translation, suggesting a distinct mechanism of action. By contrast, the TENT4 inhibitor RG7834 blocked incorporation of 5-ethynyl uridine into nascent HAV RNA, indicating that TENT4A/B function in viral RNA synthesis. Consistent with its in vitro antiviral activity (IC50 6.11 nM), orally administered RG7834 potently inhibited HAV replication in Ifnar1-/- mice, sharply reducing serum ALT, hepatocyte apoptosis, and intrahepatic inflammatory cell infiltrates. These results reveal previously unknown requirements for ZCCHC14-TENT4 in hepatovirus RNA synthesis, and suggest TENT4 inhibitors may be useful for preventing or treating hepatitis A in humans. A new generation of hepatoselective DHQ (HS-DHQ) derivatives has been developed that accumulate specifically within the liver, reducing extrahepatic drug exposure. These compounds retain activity against HAV, and likely offer an improved safety profile.

056. Differential Dynamics and Evolution of Cytomegalovirus Infection in Transplant Recipients Grafted With Organs Derived From the Same Donor

Fien Horsten, Ph.D. Seeking, *KU Leuven, Leuven, Belgium*
Sunwen Chou, *United States*
Piet Maes, Ph.D., *KU Leuven, Leuven, Belgium*
Robert Snoeck, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Graciela Andrei, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

Human herpesviruses are highly prevalent, large DNA viruses that establish lifelong latency in the host after primary infection. Human cytomegalovirus (HCMV) is a major concern in transplant patients who undergo immunosuppressive therapy, either as a consequence of primary infection, re-infection, or reactivation, causing significant morbidity and mortality. Under our translational research platform RegaVir, we analyzed the evolution of the HCMV infection in three patients who received a transplant from the same donor on the same date. The 3 patients were infected with the same HCMV strain derived from the donor as all viral isolates recovered from the 3 patients had the same UL97 protein kinase (PK) and DNA polymerase (DP) natural genetic polymorphisms, as well as the novel A505G DP substitution. This change could be linked to a natural polymorphism following phenotyping of a recombinant virus bearing this mutation. The HCMV infection evolved differently in the 3 patients: the bowel/pancreas recipient developed ganciclovir/foscarnet resistance due to the acquisition of 3 different DP mutations (V781I, E951Q, V715M), the kidney recipient had a wild-type HCMV infection that responded to HCMV antiviral therapy, and the lung recipient developed ganciclovir resistance followed by maribavir resistance due to the emergence of the L595S and T409M UL97 PK mutations, respectively. Using a capture probe library for HCMV DNA enrichment, whole-genome HCMV sequencing was performed, and data are being analyzed to evaluate the variation and short-term HCMV evolution both between-patients and within-patients.

057. Two Novel Small Chemical Compounds Blocking Herpes Simplex Virus Assembly

Julio Cesar Villalvazo Guerrero, Ph.D., *Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover-Braunschweig Site, Hannover, Germany*
Liyao Deng, Ph.D. Seeking, *Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover-Braunschweig Site, Germany*
Anne Binz, *Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover-Braunschweig Site; RESIST - Cluster of Excellence, Hannover Medical School, Hannover, Germany*
Timmy Richardo, Ph.D. Seeking, *Institute of Virology, Hannover Medical School; RESIST - Cluster of Excellence, Hannover Medical School, Hannover, Germany*
Anja Pohlmann, *Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover-Braunschweig Site; RESIST - Cluster of Excellence, Hannover Medical School, Hannover, Germany*
Buch Anna, Ph.D., *Institute of Virology, Hannover Medical School, Hannover, Germany*
Dorcas Otoo, Ph.D., *Institute of Virology, Hannover Medical School, Hannover, Germany*
Nanaji Arisetti, Ph.D., *German Center for Infection Research (DZIF), Hannover-Braunschweig Site; Helmholtz Centre for Infection Research, Braunschweig, Germany*
Konrad Wagner, Ph.D. Seeking, *German Center for Infection Research (DZIF), Hannover-Braunschweig Site; Helmholtz-Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany*
Mark Brönstrup, Ph.D., *German Center for Infection Research (DZIF), Hannover-Braunschweig Site; Helmholtz Centre for Infection Research, Braunschweig, Germany*
Martin Empting, Ph.D., *German Center for Infection Research (DZIF), Hannover-Braunschweig Site; Helmholtz-Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany*
Rudolf Bauerfeind, Ph.D., *Research Core Unit for Laser Microscopy (ReCoLa), Hannover Medical School, Hannover, Germany*
Beate Sodeik, Ph.D., *Institute of Virology, Hannover Medical School; German Center for Infection Research (DZIF), Hannover-Braunschweig Site; RESIST - Cluster of Excellence, Hannover Medical School, Hannover, Germany*

Herpes simplex viruses (HSV-1, HSV-2) cause disabling infections in newborns as well as encephalitis and eye infections at all ages, particularly in immunocompromised patients, e.g. after transplantation or during AIDS. The WHO estimates that HSV-1 has infected more than two thirds and HSV-2 about every 15th person of the world's adult population. Currently there are no licensed vaccines but acyclovir and its derivatives, that inhibit the viral DNA polymerase, are widely used in the clinic. Thus, there is an unmet need for additional drugs that target other steps of HSV infection to complement its treatment.

We screened a local library of about 19,000 small chemical compounds against HSV1(17+)Lox-GFP. Among the top compounds, PANH_135 and PANH_070 inhibited the formation of infectious virions with an IC50 of 0.6 or 10.1 μM, respectively, and a CC50 higher than 150 μM. PANH_135 inhibited DNA replication, blocked C-capsid formation, and led to overall fewer nuclear and cytoplasmic capsids. In contrast, although the capsids had recruited the large tegument protein pUL36, infection in the presence of PANH_070 led to higher amounts of nuclear and cytoplasmic capsids, suggesting that nuclear capsid egress and secondary envelopment had been inhibited. Both compounds inhibited infection of HSV-1 and HSV-2 acyclovir-resistant strains, and HSV-1 infection of murine skin ex vivo. Importantly, resynthesized compounds and some derivatives maintained their activity against HSV-1. We are currently isolating and sequencing compound-resistant HSV-1 strains, and using derivatives for proteolysis-targeting chimera technology (PROTAC) to identify the viral targets of these PANH compounds.

058. Herpesvirus-mediated Protein Citrullination as a New Target for Antiviral Therapy

Selina Pasquero, Ph.D., *Department of Public Health and Pediatric Sciences, University of Turin – Medical School, Turin, Italy*
Francesca Gugliesi, *Department of Public Health and Pediatric Sciences, University of Turin, Italy*
Gloria Griffante, *University of Piemonte Orientale, Novara, Italy*
Valentina Dell'Oste, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Matteo Biolatti, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Camilla Albano, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Greta Bajetto, *University of Turin, Turin, Italy*
Paul Thompson, *University of Massachusetts Medical School, Massachusetts, United States*
Sen Sudeshna, *University of Massachusetts Medical School, Massachusetts, United States*
Santo Landolfo, *University of Turin, Turin, Italy*
Marco De Andrea, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*

Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that remains latent in neuronal cell bodies but reactivates following a variety of stresses throughout the individual's life. In some cases, individuals can develop adverse reactions such as herpes simplex encephalitis (HSE). In this context, recent evidence suggests the involvement of HSV-1 in the etiology of Alzheimer's disease (AD). The absence of an effective vaccine and the emergence of numerous drug-resistant variants led the need to develop new antiviral agents able to tackle HSV-1 infections. In this scenario, host-targeting antivirals (HTAs) which act on host-cell factors essential for viral replication, are emerging as a promising class of antiviral compounds. Here we show that a new class of HTAs targeting peptidylarginine deiminases (PADs), a family of calcium-dependent enzymes catalyzing protein citrullination, display a potent inhibitory activity against HSV-1. Specifically, we show that inhibition of PADs-mediated citrullination suppresses HSV-1 replication in fibroblasts (HFF), epithelial (ARPE), or neuroblastoma (SHSY-5Y) cell lines. Furthermore, we show that HSV-1 infection leads to enhanced protein citrullination through transcriptional activation of three PAD isoforms: PAD2, PAD3 and PAD4. Interestingly only PAD3 specific inhibitors, CAY10727 or HF4, dramatically curbs HSV-1 replication. Finally, we defined HSV-1-induced citrullinoma that could be useful to understand how citrullination is crucial for HSV-1 replication. Overall, our results demonstrate that PAD inhibitors suppress efficiently HSV infection in vitro which may provide the rationale for their reporpusing use as a HSV-1 antivirals.

059. Combined gB (humoral) and IE1 (cell-mediated) Ad Vector CMV Vaccines Are More Effective Than Disabled CMV DISC Vaccine for cross Strain Protection Against Congenital Cytomegalovirus Disease

Alistair McGregor, Ph.D., *Texas A&M University Health Science Center, Bryan, TX, United States*
K. Yeon Choi, Ph.D., *Texas A&M University Health Science Center, Texas, United States*

Guinea pig cytomegalovirus (GPCMV) is the only small animal model for congenital CMV. A recent GPCMV DISC vaccine demonstrated the importance of neutralizing antibodies to viral glycoproteins for protection against congenital CMV. Clinical strains of HCMV are highly cell-associated and potentially evade neutralizing antibodies by limiting levels of cell free virus. In convalescent HCMV, the T cell response to IE1 protein is considered important. We verified that GPCMV encodes essential functional homologs of IE1 and IE2. GPCMV IE1 was explored as an adenovirus-based vaccine candidate (AdIE1). Guinea pig specific IFNγ ELISPOT assay to IE1 showed T cell response in vaccinated animals. AdIE1 vaccinated animals challenged with wild type GPCMV (22122 strain) showed reduced viral load in target organs but lacked full protection similar to a previous trimeric capable AdgB vaccine. Although AdgB induced high titer neutralizing antibodies, an AdgB vaccine was less effective against a novel cell associated clinical GPCMV

strain (TAMYC). The combination of AdgB and AdIE1 to target both humoral and cell-mediated response against mixed strains of GPCMV was explored in a congenital protection study. Groups of female guinea pigs were vaccinated with either AdgB, AdgB+AdIE1, or unvaccinated before mating. During 2nd trimester animals were challenged with multiple GPCMV strains (22122 and TAMYC). Only AdgB+AdIE1 vaccine group provided complete protection against congenital infection by mixed strains with no detectable virus in pup tissues. Outcome demonstrates the importance of combined immune response against these key antigens for high vaccine efficacy against congenital infection.

060. Different Epigenetic Inhibitors Targeting Chromatin Remodeling Complexes Inhibit or Activate HSV-1 Replication

Sarah Saddoris, Ph.D. Seeking, *Cornell University, Ithaca, NY, United States*
Kairui Sun, B.S., *Cornell University, NY, United States*
Luis M. Schang, D.V.M., Ph.D., *Cornell University, Ithaca, NY, United States*

Herpes simplex virus 1 (HSV-1) is a globally endemic DNA virus with a major impact on human health. HSV-1 infects epithelial cells during lytic infection and establishes latency in neurons, resulting in life-long infections. While most infections are asymptomatic, HSV-1 produces disease ranging from oral and genital lesions to stromal keratitis and encephalitis. No vaccine is available, and antivirals are unable to prevent reactivation or eliminate latent virus. During latent infection, the HSV-1 genome is assembled into stable chromatin and minimally transcribed, whereas it is assembled into highly dynamic chromatin and highly transcribed in lytic infections. It is unclear how HSV-1 transitions from static chromatin during latency to highly dynamic chromatin during reactivation, but epigenetic drugs have been proposed as potential anti-HSV-1 agents. We tested six small molecule modulators of chromatin remodeling complexes on HSV-1 lytic replication in culture. Four increased replication and only two inhibited it. The results are consistent with the chromatin remodeling complex GBAF inhibiting HSV-1 replication and PBAF and NORC promoting it. To start exploring whether these complexes act on HSV-1 chromatin, we performed immunofluorescence for selected subunits. Three common and one unique subunit from each cBAF, PBAF, and GBAF were enriched in the herpes nuclear domains (HND). While the localization of the subunits of PBAF and cBAF to HND were largely unaffected by the epigenetic modulators, that of the unique GBAF subunit was highly sensitive. Our results suggest that GBAF complexes inhibit HSV-1 replication via direct mechanisms on the viral chromatin.

061. POM-L-BHDU is a Highly Potent Prodrug of L-Dioxolane Bromovinyl Uridine That Prevents Varicella Zoster Virus Spread Topically In Skin Organ Culture and Orally In NuSkin Mice

Megan Lloyd, Ph.D., *SUNY Upstate Medical University, Syracuse, NY, United States*
Uma Singh, *The University of Georgia, GA, United States*
Yugandhar Kothapalli, *The University of Georgia, Athens, GA, United States*
Vidya Annavarapu, *The University of Georgia, Athens, GA, United States*
Michael Bartlett, *The University of Georgia, Athens, GA, United States*
Chung Chu, *The University of Georgia, Athens, GA, United States*
Jennifer Moffat, Ph.D., *Upstate Medical University, Syracuse, New-York, United States*

Potent and safe antivirals are needed to treat herpes zoster, caused by varicella zoster virus (VZV). We showed that L-BHDU (β-L-1-[5-(E-2-bromovinyl)-2-(hydroxymethyl)-1,3-(dioxolan-4-yl)] uracil) prevented VZV spread in humanized SCID mice, but it required continuous dosing in Alzet pumps. To improve its pharmacologic properties, we synthesized a prodrug, POM-L-BHDU, with a bis(pivaloyloxymethyl) group. POM-L-BHDU was effective against VZV but not cytotoxic in ARPE-19 cells (EC50=0.036 μM, CC50>100 μM). Next, we evaluated POM-L-BHDU for its effectiveness against VZV in skin and mice. Adult human skin was obtained from reduction mammoplasties, and 1-cm2 pieces were placed on NetWells at the air-media interface (skin organ culture, SOC) or implanted subcutaneously in athymic nude mice (NuSkin model). Skin was inoculated with VZV-ORF57-Luc by scarification and virus spread was measured by bioluminescence imaging. POM-L-BHDU formulated in cocoa butter (0.2%) prevented VZV spread in SOC treated topically and was not toxic. In NuSkin mice, POM-L-BHDU significantly reduced VZV spread when administered subcutaneously at 22.4 mg/kg QD or orally at 45, 22.4 and 11.3 mg/kg QD. POM-L-BHDU was well tolerated in mice. Mice were given POM-L-BHDU orally and plasma was analyzed by LC-MS/MS. The half-life of the L-BHDU metabolite was 5.6 h. Studies are ongoing to compare absorption rates between POM-L-BHDU and L-BHDU and to determine distribution in mouse organs. Here, we show that POM-L-BHDU is safe and effective in culture and mice, meeting a need for better antivirals. This work was supported in part by NIAID DMID contract HHSN272201700030I to JFM.

062. Identification of 27-Hydroxycholesterol Synthetic Analogs as a Novel Class of Anti-Herpes Simplex Virus Antivirals

Andrea Civra, Ph.D., *University of Turin - Department of Clinical and Biological Sciences, Orbassano, Turin, Italy*
Matteo Costantino, *University of Turin - Department of Clinical and Biological Sciences, Orbassano, Turin, Italy*
Emanuele Carosati, *University of Trieste, Italy*
Giuseppe Poli, *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*
Maura Marinozzi, *University of Perugia, Italy*
David Lembo, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*

Genital herpes, most frequently caused by herpes simplex virus type 2 (HSV-2) infection, is one of the most prevalent sexually transmitted diseases. There is neither a cure nor a preventive treatment for genital herpes; the current rationale for the treatment of HSV-2 infection involves nucleoside analogs to suppress reactivation. 27-hydroxycholesterol (27OHC) is an endogenous 27-carbon molecule derived from cholesterol oxidation, and recently emerged as a broad-spectrum host targeting antiviral. In this study, we screened selected members of an in-house synthesized library of 27OHC analogues for their activity against HSV-2. In a first set of experiments, we tested the antiviral efficacy of 27OHC and a library of 17 synthetic analogues by plaque reduction assay. Molecules with the highest selectivity indexes (SIs) were selected and their antiviral activity was confirmed by virus yield reduction assay. The step of viral replication inhibited and the putative cellular target of these molecules were investigated by transmission electron microscopy and indirect immunofluorescence. The screening of the panel against HSV-2 culminated in the identification of the two hit compounds, named PFM067 and PFM069, endowed 50% effective concentrations (EC50) in the low micromolar range, and characterized by selectivity indexes (SIs=CC50/EC50) above 100. Moreover, the results obtained showed the interesting ability of the novel derivatives to inhibit cell-to-cell fusion induced by HSV-2, by sequestering viral glycoproteins in the Golgi compartment. Taken together, these results point to PFM067 and PFM069 as promising chemical scaffolds for the development of novel antivirals.

100. A Gut-Immune Model System Using Human Intestinal Enteroids to Identify Antivirals Targeting Enteric Viruses and the Host Immune Response

Jana Van Dycke, Ph.D., *KU Leuven – Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Nanci Santos-Ferreira, M.S., *KU Leuven, Rega Institute, Department of Microbiology, Immunology and Transplantation, Leuven, Belgium*
Mieke Gouwy, *KU Leuven – Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Molecular Immunology, Leuven, Belgium*
Paul Proost, *Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Molecular Immunology, Leuven, Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Joana Rocha-Pereira, Ph.D., *KU Leuven, Department of Microbiology, Immunology & Transplantation, Rega Institute, Laboratory of Virology & Chemotherapy, Leiden, Belgium*

Human intestinal enteroids (HIEs) have an intermediate complexity, situating between cell lines and animal models, hence being a transformative tool in virology and antiviral research. Before HIE cultures, the cultivation of enteric viruses such as human norovirus (HuNoV) was at large not possible, which hampered the development of therapeutics against HuNoV infections. Infection of differentiated HIEs seeded on transwell inserts in multiwell plates with HuNoV GII.4 yielded a 2log increase over input in viral loads at two days post infection. The effect of reference antiviral 2'-C-Methylcytidine was confirmed for validation purposes. The use of transwell inserts provides access to both the apical and basolateral side of cultures, hence allowing us to study HuNoV shedding and thus indirectly transmission, for the first time. Besides the multiple intestinal cell subtypes present in HIEs, immune cells are also important and closely interact with enteric viruses in the small intestine. Therefore, we are now adding human PBMC-derived macrophages to cultures, apically and/or basolaterally. The infection and cell-to-cell viral spread is being studied by RT-qPCR and confocal imaging; cytokine secretion will be determined via ELISA. We can then identify inhibitors that act on the host immune response, in addition to those targeting the virus. Cellular toxicity will be evaluated via an ATP-based luminescent cell-viability assay and confocal imaging. In the future, we plan to extend the use of this model to other (gastro)enteric viruses. Additionally, we aim to study the interaction of viruses with the different intestinal/immune cell types via RNAseq and immunohistochemistry.

101. High-content Antiviral Screening Assay for Enteric Viruses Using Human Intestinal Organoids

Joana Rocha-Pereira, Ph.D., *KU Leuven, Department of Microbiology, Immunology & Transplantation, Rega Institute, Laboratory of Virology & Chemotherapy, Leiden, Belgium*
Nanci Ferreira, M.S., *Rega Institute for Medical Research, Leuven, Belgium*
Fatma Masmoudi, M.S., *Charles River Discovery, Netherlands*
Jeroen Esselink, M.S., *Charles River Discovery, Leiden, Netherlands*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Marijn Vlaming, Ph.D., *Charles River Discovery, Leiden, Netherlands*
Ludovico Buti, Ph.D., *Charles River Discovery, Leiden, Netherlands*

The development of human intestinal organoid cultures, non-transformed 3D-organised human intestinal aggregates that recapitulate the complex physiology of the human intestine, represents an excellent opportunity to push antiviral drug discovery to the next level. Using these 3D-cultures, hard-to-cultivate enteric viruses such as human norovirus can now be studied in vitro, while aspects of the replication of enteroviruses such EV-A71 can be dissected in complex cultures containing multiple intestinal cell types. This opens the door to development of therapeutics for these viruses, which are currently not available.

The aim of this work was to establish gut organoids as a drug screening platform for antiviral agents. To that end, we infected an iPSC-derived human intestinal organoids (HIOs) with EV-A71. A 384-well screening assay was setup using cell markers (DAPI and Phalloidin) and an antibody for the dsRNA intermediate as virus marker. Hence, the assay is broadly applicable to all (+)ssRNA viruses. Image analysis shows EV-A71 MOI to be proportional to the percentage of infected HIOs. The antiviral activity of reference antivirals such as 2'-C-Methylcytidineand and rupintrivir was confirmed by RT-qPCR in 384-well format. Further analysis is ongoing to validate the use of HCA readout in screening for compound with antiviral activity. Overall, the high-content antiviral screening approach is potentially applicable to multiple viruses, opening the door to a first-time large compound screening campaign using gut organoids.

103. Identification of Dctn6, a Druggable Host Factor Exploited by Enterovirus-71 During Its Infection Cycle

Elijah Chen, Ph.D., *NUS, Singapore*

Enterovirus A71 (EV-A71) is one of the main aetiological agents of Hand, Foot, and Mouth Disease (HMFD). Although the symptoms of HFMD are generally mild and self-limiting, EV-A71-associated HFMD is linked to more severe complications involving the neuronal and cardiac systems, which can be life threatening. There are no effective antivirals or vaccines approved on a global scale for HFMD, with current treatment limited to alleviation of symptoms. EV-A71 is mainly transmitted via the oral-faecal route: upon ingestion, EV-A71 replicates effectively in the gut epithelium and underlying intestinal muscle layer. At the neuromuscular junctions, EV-A71 then enters the peripheral nervous system and employs retrograde axonal transport to invade the central nervous system and reach the brainstem. However, the molecular mechanisms involved in EV-A71 neuroinvasion and neuropathogenesis are unclear. To address this knowledge gap, we screened an siRNA library to identify host factors essential for EV-A71 infection in NSC-34 cells, a murine motor neuron-like cell line. Among the hits, Dctn6 was selected for further investigation. Dctn6 is a component of the dynactin complex, the cofactor for the microtubule motor, dynein. Given dynein's role in retrograde intracellular transport, the involvement of Dctn6 in EV-A71 replication is not surprising. Inhibition of the dynein motor by several drugs with distinct modes of action suppressed EV-A71 infection effectively, suggesting that the dynein motor can be targeted by small molecules to control EV-A71 infection. Additional experimental approaches suggested that Dctn6 plays a role during the entry step of the infection cycle.

104. Norovirus NS1-2 as a Target for the Computer-aided Identification of Novel Antiviral Agents

Salvatore Ferla, Ph.D., *Swansea University Medical School, Swansea, United Kingdom*
Joshua Nadeem, B.S., *United Kingdom*
Nanci Santos-Ferreira, M.S., *KU Leuven, Rega Institute, Department of Microbiology, Immunology and Transplantation, Leuven, Leuven, Belgium*
Joana Rocha-Pereira, Ph.D., *KU Leuven, Department of Microbiology, Immunology & Transplantation, Rega Institute, Laboratory of Virology & Chemotherapy, Leiden, Belgium*
Marcella Bassetto, Ph.D., *Department of Chemistry, College of Science and Engineering, Swansea University, Swansea, Swansea, United Kingdom*

Human norovirus is the first cause of food-borne disease worldwide, leading to extensive outbreaks of acute gastroenteritis, causing severe and life threatening chronic diarrhoea in immunocompromised patients . No specific vaccines or antivirals to treat or prevent this infection are currently available, despite there being a clear medical need. Direct acting antivirals have shown to be successful in controlling viral infections, but besides the polymerase and protease no other norovirus non-structural protein has been exploited as antiviral target. Th norovirus NS1-2 (p48) has an N-terminal portion that is inherently disordered and likely interacts with multiple cellular factors to facilitate genome replication. However, its C-terminal part is more conserved, does not tolerate insertions and shows the presence of a putative catalytic triad analogous to that of papain-like thiol peptidases. This C-terminal domain is a potentially druggable target, thus far unexploited. Using an in-silico protein threading approach, different 3D-models of norovirus NS1-2 protein were constructed. The most reasonable model was then used to screen in-silico a commercial compound library, to select potential inhibitors of the peptidase activity. The best virtual hits identified were purchased and their antiviral activity explored in a cellular assay using the murine norovirus as a surrogate. One small molecule was shown to protect infected cells from virus-induced cytopathic assay with an EC50 of ~43 μM. In this presentation, the in-silico studies will be discussed, together with the preliminary results obtained in the cell-based antiviral assays, and future directions of this work.

200. 1 Prophylactic and Therapeutic Efficacy of a Novel Brain-penetrant Antiviral in 2 Lethal Mouse Models of Venezuelan and Eastern Equine Encephalitis

Colleen Jonsson, Ph.D., *University of Tennessee Health Science Center, Memphis, TN, United States*
Jennifer Golden, Ph.D., *Department of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, Wisconsin, United States*
Bernd Meibohm, Ph.D., *University of Tennessee Health Science Center, Memphis, TN, United States*
Dong-Hoon Chung, Ph.D., *University of Louisville, Louisville, KY, United States*

Venezuelan and eastern equine encephalitis viruses (VEEV and EEEV, respectively) are mosquito-borne, neuroinvasive human pathogens for which no FDA-approved therapeutic exists. Besides the biothreat posed by these viruses when aerosolized, arthropod transmission also presents significant health risks. Herein, we report the discovery of a novel 2-pyrrolidinoquinazolinone scaffold, efficiently synthesized in 2-5 steps, whose structural optimization resulted in profound antiviral activity. The lead quinazolinone, BDGR-49, potently reduced cellular VEEV and EEEV titers by > 7-log at 1 43 mM and exhibited suitable intravenous and oral pharmacokinetic profiles in BALB/c mice to achieve excellent brain exposure. Outstanding in vivo efficacy was observed in several lethal, subcutaneous infection mouse models using an 8-day dosing regimen. First, prophylactically administered BDGR-49 at 25 mg kg-1 46 per day fully protected against a 10xLD50 VEEV TrD47 challenge in BALB/C mice. Similarly, we observed 70% protection when 10xLD50 EEEV FL93- 939 infected C57BL/6 mice were treated prophylactically with BDGR-49 at 50 mg kg-1 48 per day. Last, we observed 100% therapeutic efficacy when mice, challenged with 10xLD50 VEEV TrD, were dosed at 48 hours post-infection with BDGR-49 at 25 mg kg-1 50 per day, and mouse brain viral titers at hours post-infection were reduced to values near the limit of detection. Collectively, these results underscore the substantial development potential of a well-tolerated, brain-penetrant lead compound that shows promise in preventing and treating encephalitic alphavirus disease.

201. Antiviral Effect of Atovaquone against Chikungunya and Zika Virus in Aedes aegypti Mosquitoes by Tarsal Exposure via the Mosquito Legs

Leen Delang, Ph.D., *Rega Institute, University of Leuven, Leuven, Belgium*
Lanjiao Wang, Ph.D., *Rega Institute for Medical Research, KU Leuven, Belgium*
Aboubakar Sanon, M.S., *Rega Institute, University of Leuven, Leuven, Belgium*
Rana Abdelnabi, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Zakiyatul Khoiriyah, B.S., *Wageningen University, Wageningen, Netherlands*

The anti-malarial drug atovaquone was recently shown to inhibit the replication of several arboviruses, such as chikungunya virus (CHIKV) and Zika virus (ZIKV), in mammalian cells. Furthermore, atovaquone was able to block Plasmodium parasite transmission by Anopheles mosquitoes when mosquitoes were exposed to atovaquone by resting with their legs on a treated surface (i.e. tarsal exposure). We therefore evaluated whether tarsal exposure to atovaquone could inhibit arbovirus replication in Aedes aegypti mosquitoes.

Atovaquone exerted a dose-dependent antiviral effect against CHIKV and ZIKV in both Vero and mosquito C6/36 cells. CHIKV and ZIKV infectious titers were reduced with ~3 log10 in Vero cells at a concentration of 5 µM and 0.6 µM, respectively. Exposure to atovaquone up to 100 µmol/m2 did not affect the egg laying and hatching rate of mosquitoes. No effect on mosquito survival was observed upon exposure to 25 µmol/m2, whereas 100 µmol/m2 slightly affected survival starting from day 10. To evaluate the antiviral effect, female mosquitoes were exposed to 100 µmol/m2 atovaquone for 1 h, after which they were infected via artificial blood meal. Two independent experiments showed that atovaquone did not decrease CHIKV infection and dissemination rates in Ae. aegypti, but reduced CHIKV transmission in mosquito saliva (transmission rate of 0% vs. 19% for treated and untreated groups). In contrast, no significant antiviral effect against ZIKV was observed in mosquitoes. In conclusion, atovaquone might exert both anti-CHIKV and anti-malarial activities in mosquitoes via tarsal exposure to a treated surface, although the anti-CHIKV effect seems less pronounced.

202. Antiviral Strategies for Encephalitic Viral Diseases

Karin Peterson, Ph.D., *Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States*
Durbadal Ojha, Ph.D., *Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA, MT, United States*

Viral-induced encephalitis is a rare, yet serious, neurological disease that can be life-threatening. Several different types of viruses can cause viral encephalitis including Orthobunyaviruses, Flaviviruses, Retroviruses, and Herpesviruses. Currently, there are limited therapeutic options for the treatment of viral encephalitis other than palliative care. One of the confounding factors with viral encephalitis is that infection is often not determined until hospitalization due to clinical signs, at which point the virus has crossed the blood brain barrier (BBB) and infected the CNS. Thus, a promising antiviral must cross the BBB and be effective at limiting virus replication, without causing damage to neurons themselves. We have focused on using neuronal cell lines including human neural stem cells (hNSCs), human cerebral organoids, and appropriate mouse models to validate antivirals against Orthobunyaviruses and Flaviviruses. We identified nucleoside analogs (Molnupiravir) as well as small molecule inhibitors (Rottlerin, Thapsigargin, etc.) that effectively inhibit virus replication, virus-induced damage in neurons, and reduced virus-induced pathogenesis in organoid and mouse models for encephalitic Orthobunyaviruses and Flaviviruses.

203. Dengue Virus Infection and Dissemination Are Disrupted in Aedes aegypti Mosquitoes Exposed to JNJ-A07 in the Bloodmeal

Ana L. Rosales Rosas, M.S., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Lanjiao Wang, Ph.D., *Rega Institute for Medical Research, KU Leuven, Belgium*
Suzanne Kaptein, Ph.D., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Olivia Goethals, *Global Public Health R&D, Janssen Pharmaceutica NV, Beerse, Belgium*
Leen Delang, Ph.D., *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*

Dengue virus (DENV) is the most widespread mosquito-borne virus worldwide; however, no antiviral therapies are available yet. The pan-serotype DENV inhibitor (JNJ-A07) has shown potent activity in a mouse model (PMID:34616043). The concept behind prophylaxis is that a drug prevents dissemination of the virus in the human body after a mosquito bite. In addition, DENV-infected mosquitoes might ingest a drug during blood feeding from a

drug-treated patient. Preliminary findings demonstrated that a DENV-infectious bloodmeal spiked with JNJ-A07 blocked DENV infection and dissemination in mosquitoes. Here, we assessed the antiviral activity of JNJ-A07 when delivered to Aedes aegypti mosquitoes via the bloodmeal in different administration regimens: pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP). When JNJ-A07 (2 μM) was provided 6 days prior a DENV-infectious bloodmeal in a PrEP scenario, JNJ-A07 blocked DENV infection in mosquitoes as determined by the reduced number of infected mosquito bodies (infection rate) at day 7 post infection (0% vs control:55%). Furthermore, when the bloodmeal containing JNJ-A07 (2 μM) was given to the mosquitoes 6 days after a DENV-infectious bloodmeal in a PEP scenario, the ongoing infection in the mosquitoes was disrupted. A decreased infection rate (57% vs control:76.2%) and lower virus dissemination to mosquito secondary organs (13.4% vs control:73.1%) could be observed in mosquitoes that ingested JNJ-A07. Our results indicate that JNJ-A07 retains its potent antiviral activity in mosquitoes when ingested during blood feeding in different scenarios.

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204. Development of Proximity-based Antivirals: Mechanisms Beyond Targeted Protein Degradation

Priscilla Yang, Ph.D., *Stanford University School of Medicine, Stanford, California, United States*
Zhengnian Li, Ph.D., *Stanford University School of Medicine, Stanford, California, United States*
Lu-Ning Wang, Ph.D., *Stanford University School of Medicine, Stanford, California, United States*
Han-Yuan Liu, Ph.D., *Stanford University School of Medicine, Stanford, California, United States*
Nathanael Gray, Ph.D., *Department of Chemical and Systems Biology, Chem-H, Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, United States*

Small molecules with proximity-based pharmacology act by inducing proximity between biomolecules, most typically protein-protein interactions. For example, small molecule glues and bifunctional degrader molecules stabilize interactions between target proteins and E3 ubiquitin ligases to induce target degradation and are currently the most widely known class of proximity-based agents. Beyond targeted protein degradation, however, there remains, significant untapped potential to develop direct-acting antivirals that capitalize on induced proximity to interfere with normal viral protein function(s). Recruitment of isomerases such as FKBP12 by macrocycles such as FK506 and cyclosporine to inhibit challenging targets such as the phosphatase calcineurin are examples of how nature exploits this approach. Proximity-based pharmacology may be especially useful against viral proteins that lack enzymatic function. To interfere with normal oligomerization of the dengue virus core protein, we synthesized bifunctional small molecules bearing a validated core protein ligand linked covalently to SLF, a validated ligand of the proline isomerase FK506-binding protein 12 (FKBP12). Screening of these small molecules allowed identification of compounds that exhibit concentration-dependent inhibition of dengue virus in cell culture via a mechanism that requires binding to FKBP12. Experiments to establish and validate the mechanism of these agents and to develop other proximity-based approaches for antiviral intervention are underway.

205. Discovery and Synthesis of 1,2,4-oxadiazole Derivatives as Novel Inhibitors of Zika Virus Infection

Yun Young Go, D.V.M., Ph.D., *City University of Hong Kong, Hong Kong (SAR China)*
Sangwoo Nam, *Korea Research Institute of Chemical Technology, Daejeon, South Korea*
Hyo Gyeong Na, *Korea Research Institute of Chemical Technology, Daejeon, South Korea Eun Hye Oh, Korea Research Institute of Chemical Technology, Daejeon, South Korea Eunhye Jung, Korea Research Institute of Chemical Technology, Daejeon, South Korea*
Yeon Hee Lee, *Korea Research Institute of Chemical Technology, Daejeon, South Korea*
Eun Ju Jeong, *Korea Research Institute of Chemical Technology, Daejeon, South Korea a*
Yu-Da Ou, *Nanjing Agricultural University, Nanjing, China*
Bin Zhou, *Nanjing Agricultural University, Nanjing, China*
Sunjoo Ahn, *Korea Research Institute of Chemical Technology, Daejeon, South Korea*
Jin Soo Shin, *Korea Research Institute of Chemical Technology, Daejeon, South Korea*
Soo Bong Han, *Korea Research Institute of Chemical Technology, Daejeon, South Korea*

Zika virus (ZIKV), an arbovirus of the Flaviviridae family, reemerged as a significant public health concern due to its association with congenital abnormalities and severe neurological sequelae. Currently, there is an unmet need for developing effective therapeutic approaches that can efficiently treat ZIKV infections on time. In this study, we identified a series of 1,2,4-oxadiazol derivatives that possess antiviral activity against in vitro ZIKV infection via a phenotypic screening of a chemical library composed of 10,000 small molecules. Subsequently, 28 new derivatives were designed, synthesized, and evaluated for their antiviral activities against ZIKV infection. Among these compounds, 4-(5-phenyl-1,2,4-oxadiazol-3-yl)-N-(pyridin-3-ylmethyl)aniline (5d) was discovered with potent antiviral activity and high selectivity against ZIKV infection. Furthermore, the structure-activity-relationship analysis indicated that benzyl substitution on the aniline nitrogen improves potency while providing better drug-like properties. Notably, 5d exhibited antiviral activity against various flaviviruses, including dengue and Japanese encephalitis viruses, indicating its potential as a lead compound for the generation of 1,2,4-oxadiazol derivatives with broad-spectrum anti-flaviviral properties.

206. Efficient Incorporation of 2′-Fluoro,2′-Bromouridine Triphosphate Inhibits Yellow Fever Virus Polymerase Selectively

Calvin Gordon, Ph.D. Seeking, *Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada*
Julia Lecher, Ph.D., *Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Georgia, United States*
Franck Amblard, Ph.D., *Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, United States*
Raymond Schinazi, Ph.D., *Center for ViroScience and Cure, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia, United States*
Matthias Götte, Ph.D., *Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada*

Yellow fever virus (YFV) is a mosquito-borne flavivirus closely related to dengue and zika virus. Despite an effective vaccine, YF outbreak and infection is persistent throughout South America and Africa. Even more concerning, severe YFV infection has a case fatality rate of 50%, and no antiviral therapies currently exist. Nucleoside analogs represent an attractive class of therapeutics because they target the viral RNA-dependent RNA polymerase (RdRp). Sofosbuvir, a 2′-modified halogen nucleoside prodrug approved for the treatment of hepatitis C (HCV), has demonstrated potent anti-YFV activity in vitro, but was only effective in vivo when administered prophylactically. Recently a non-toxic 2′-fluoro,2′-bromouridine prodrug (compound A), was found to display in vitro and in vivo activity against YFV. Here, we expressed and purified the RdRp of YFV and characterized the biochemical properties of the active nucleoside triphosphates (NTP) forms of both sofosbuvir and compound A. In their active NTP form, 2′-fluoro,2′-methyluridine (sofosbuvir) and 2′-fluoro,2′-bromouridine (compound A) are incorporated with similar efficiency. Once incorporated, both 2′-modified halogen nucleotide analogs inhibit RNA synthesis via chain termination. Further investigation of the 2′-fluoro,2′-bromouridine-TP against the human mitochondrial RNA polymerase (h-MtRNAP) revealed that the NTP of compound A is poorly incorporated, aligning with previous reports of low cytotoxicity. This evaluation of the 2′-fluoro,2′-bromouridine-TP alongside sofosbuvir-TP provides insight into Compound A antiviral efficacy against YFV, supporting future preclinical development.

207. Viral Kinetics During Acute Chikungunya Virus Infection and the Potential for Antiviral Treatment

Hugh Watson, Ph.D., *Evotec ID (Lyon), Lyon, Rhone, France*
Nicolas Voirin, Ph.D., *Epimod, Rhone, France*
Veasna Duong, M.D., Ph.D., *Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
Sowath Ly, M.D., Ph.D., *Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
Tey Putita Ou, Ph.D., *Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
Sopheak Sorn, Ph.D., *Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
Sreymom Ken, Ph.D., *Institut Pasteur du Cambodge, Phnom Penh, Cambodia*
Marie Mandron, Ph.D., *Evotec ID (Lyon), Lyon, France*

Background: Population sampling in patients with suspected acute chikungunya infection suggests that patients typically exhibit viraemia up to 5 or 6 days after symptom onset. Viral kinetics data in individual subjects will permit more reliable modelling of the impact of an antiviral treatment.

Methods: Patients with acute chikungunya infection were followed for 10 days with sampling every 2 days for viraemia and IgM. Viral load was determined by RT-PCR and anti-CHIKV IgM by ELISA. Patients' symptoms and C-reactive protein (CRP) were also followed. Observed kinetics and clinical presentation were described.

Results: 29 subjects (14 adults and 15 children, aged >2 and <18 years) were included on average 2.6±3.2 days after onset of symptoms. Viral load ranged from 1.091×103 to 8.233×109 and the peak was observed on average 2.7±3.2 days after symptom onset. IgM and IgG became detectable on average 5.4±2.2 days and 8.5±2.1 days after onset of symptoms respectively. Most frequent symptoms at first visit were fever (90%), arthralgia (79%), fatigue (76%) and headache (69%). Clinical presentation of symptoms was not statistically different between adults and children. A tendency toward higher viral load (p=0.046) and longer duration to first detectable IgG (p=0.047) was observed among children compared to adults.

Conclusion: Within-subject viraemia profiles were largely consistent with those derived from population sampling. Further modelling of viral and immunologic kinetics is ongoing and will permit estimation of the total duration of viraemia from the time of infection and the impact of future antiviral agents on exposure to circulating CHIKV during the acute infection.

208. Identification of Novel Regulatory Sites of Alphavirus Non-structural Protein 2 Activity Via Alphafold2 Informed Mutagenesis

Jack Sears, Ph.D. Seeking, *University of North Carolina - Chapel Hill, Department of Microbiology and Immunology, Chapel Hill, NC, United States*
Jennifer Loome, Ph.D. Seeking, *University of North Carolina - Chapel Hill, Department of Microbiology and Immunology, North Carolina, United States*
Paul Sylvester, Ph.D., *University of North Carolina - Chapel Hill, Department of Microbiology and Immunology, Chapel Hill, North Carolina, United States*
Rebekah Dickmander, Ph.D. Seeking, *University of North Carolina - Chapel Hill, Department of Microbiology and Immunology, Chapel Hill, NC, United States*
Konstantin Popov, Ph.D., *University of North Carolina Chapel Hill Division of Chemical Biology and Medicinal Chemistry, Chapel Hill, North Carolina, United States*
Alexander Tropsha, Ph.D., *University of North Carolina Chapel Hill Division of Chemical Biology and Medicinal Chemistry, Chapel Hill, North Carolina, United States*
Eric Merten, Ph.D. Seeking, *University of North Carolina Chapel Hill, School of Pharmacy - Center for Integrative Chemical Biology and Drug Discovery, Chapel Hill, North Carolina, United States*
Kenneth Pearce, Ph.D., *University of North Carolina Chapel Hill, School of Pharmacy - Center for Integrative Chemical Biology and Drug Discovery, Chapel Hill, NC, United States*
Jamie Arnold, Ph.D., *University of North Carolina - Chapel Hill, Department of Microbiology and Immunology, Chapel Hill, NC, United States*
Mark Heise, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States*
Nathaniel Moorman, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States*

Alphaviruses such as Chikungunya (CHKV) and Venezuelan equine encephalitis virus are arboviruses that are significant pandemic and bioterror threats. Despite the threats posed by alphaviruses, there are currently no approved antiviral therapies or vaccines available for the treatment or prevention of any alphavirus infection. Alphaviruses require protease and helicase activity from non-structural protein 2 (nsP2) for replication. The conserved, multifunctional, and essential nature of nsp2 in the alphavirus replication cycle makes it an ideal target for broadly acting antiviral therapeutics. To define potential drug targets in conserved domains within nsP2 we used Alphafold2, an AI-based platform that accurately predicts protein structure, and FTMap, a computational mapping server that identifies binding hot spots of macromolecules, and identified multiple novel highly conserved pockets on the surface of the nsp2 protease domain. We then generated a series of point mutations in each pocket in the CHKV genome and tested their effects on virus replication. Importantly, the mutations were designed to not alter the overall structure of nsP2. Our data show that two of the pockets are required for efficient viral RNA infectivity and replication and protease activity. Mutating these pockets in other alphaviruses similarly reduced virus replication. Several mutations differentially impacted cleavage kinetics of known nsP2 substrates, suggesting a role for these sites in substrate recognition. These data display the utility of using Alphafold2-informed modeling to identify novel target sites and suggest this approach may be broadly applicable to antiviral drug development efforts.

209. Molecular Architecture of Chikungunya Virus Replication Complexes for Antiviral Drug Discovery

Yaw Bia Tan, Ph.D. Seeking, *Nanyang Technological University, Singapore*
Dahai Luo, Ph.D., *Nanyang Technological University, Singapore*
David Chmielewski, Ph.D., *Stanford University, Palo Alto, CA, United States*

Chikungunya virus (CHIKV) is a re-emerging mosquito-borne infectious disease affecting millions of people worldwide without FDA-approved drugs. CHIKV can progress to chronic polyarthritis that may last for years post-infection. CHIKV has its mRNA-like RNA genome expressing four nonstructural proteins (nsP1-4) as replication complex (RC) with enzymatic functions and interacting with host factors. However, how these multi-component RCs assemble to synthesize and transport the RNA at the replication site in the RNA viruses are unknown. Combining cryo-electron microscopy and tomography, molecular architectures of CHIKV RC within CHIKV-infected human cell and in vitro reconstituted RC core complex were determined. The membrane-embedded core complex was determined to 2.8 Å and fits well into the 8 Å cellular RC sitting at the spherule neck. The heteromeric core complex consists of a copy of nsP2 helicase-protease docked above an active nsP4 RdRp that sits in the central pore of dodecameric nsP1 ring with bound ligands, like RNA and NTP. Interestingly, the active CHIKV RC architecture has an extra cytoplasmic ring density above its core complex, likely occupied by nsP3, viral RNA and host factors. This informed the first high-resolution RC architecture among the RNA viruses. This major advancement proposed valuable molecular insights on the CHIKV replication process comprising of multiple attractive targets for antiviral drug discovery.

210. Potent Primate-specific Pan-dengue Inhibitors

Chin Piaw Gwee, M.S., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*
Tommaso Felicetti, Ph.D., *Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia, Italy*
Wing Ki Kitti Chan, Ph.D., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*
Wint Wint Phoo, Ph.D., *Center for Global Health Discovery, Duke-NUS Medical School, Singapore,*
Min Jie Alvin Tan, Ph.D., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*
Sylvie Alonso, Ph.D., *Infectious Diseases Translational Research Programme, Department of Microbiology & Immunology, Yong Loo Lin School of Medicine, Singapore*
Giuseppe Manfroni, Ph.D., *Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia, Perugia, Italy*
Subhash Vasudevan, Ph.D., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*

Dengue virus (DENV), a mosquito-borne flavivirus, continues to be a major public health threat in many countries and there are no antiviral therapeutics available. In this work, we discovered a sulfonyl anthranilic acid (SAA) derivative of the 2,1-benzothiazine 2,2-dioxide core that was previously used to develop DENV NS5 polymerase inhibitors. Of the 38 SAA derivatives, several exhibited potent inhibitions against DENV infection in the cell-based assay, but surprisingly did not inhibit in vitro DENV NS5 polymerase activity. Notably, compound 64 showed EC50 values in the range of 0.3 to 0.6 μM against the four dengue serotypes (DENV-1-4). Time of addition assay revealed that analogue 64 is a post-entry replication inhibitor that appears to be specific for cells of primate origin, implicating a host target. We have taken a high throughput proteomic approach, Cellular Thermal Shift Assay coupled to Mass Spec (MS-CETSA), to identify potential host targets that are currently being validated in gene knock out assays to elucidate the mechanism of action for compound 64. Compound 64 could serve as a lead for more potent inhibitors against the target since it also shows similar antiviral efficacy against other RNA viruses that have been tested.

211. Reduced Dengue Virus Replication Upon Oral Exposure of Aedes Aegypti Mosquitoes to the antiviral Nucleoside Analog 7DMA

Febrina Meutiawati, M.S., *Radboud University Medical Center, Jakarta Selatan, DKI Jakarta, Indonesia*
Benoit Besson, Ph.D., *Radboud University Medical Center, Gelderland, Netherlands*
Sameera Patel, Ph.D., *TropIQ, Nijemegen, Gelderland, Netherlands*
Gijs J. Overheul, B.S., *Radboud University Medical Center, Nijmegen, Gelderland, Netherlands*
Denise van de Kamer, M.S., *Radboud University Medical Center, Nijmegen, Gelderland, Netherlands*
Koen J. Dechering, Ph.D., *TropIQ, Nijmegen, Gelderland, Netherlands*
Ronald P. van Rij, Ph.D., *Radboud University Medical Center, Nijmegen, Gelderland, Netherlands*

Arthropod-borne (arbo)viruses are a non-taxonomic group of viruses that are transmitted by arthropod vectors, primarily mosquitoes, and an important cause of human disease worldwide. Dengue virus (DENV) is the arbovirus with the largest global burden, but an effective vaccine and specific treatment are still lacking. An alternative approach to reduce DENV disease burden is to block virus transmission by the Aedes vector mosquitoes. Here, we explore the potential of small molecules known to inhibit DENV replication in mammalian cells as an approach to block virus transmission in mosquitoes. We selected 15 compounds from literature and confirmed antiviral activity of ten compounds in HeLa cells containing a DENV2 replicon and an additional four compounds using wild type DENV2 infection. To assess the antiviral activity in mosquito cells, we established a DENV subgenomic replicon in Aedes albopictus U4.4 cells. Three compounds, 7-deaza-2'-C-methyladenosine (7DMA), erythrosin-B, and PF-06409577, significantly reduced replication of the DENV1 replicon in U4.4 cells and replication of wildtype DENV2 in U4.4 cells and the Aedes aegypti Aag2 cell line. Upon oral exposure of adult female Ae. aegypti mosquitoes, 7DMA significantly reduced DENV2 replication without apparent toxicity. Altogether, our results provide proof-of-principle that small molecule antivirals can be used to inhibit DENV replication in Ae. aegypti, which may serve as a basis to develop intervention strategies to inhibit arbovirus transmission.

212. Screening and Phytochemical Characterization of Endemic Plants from Madagascar and Reunion Island for Antiviral Activity Against Zika and Dengue Virus

Chaker El Kalamouni, Ph.D., *PIMIT, Processus Infectieux en Milieu Insulaire Tropical, Université de La Réunion, INSERM U1187, CNRS 9192, IRD 249, plateforme CYROI 97490 Sainte-Clotilde, La Réunion, France*
Bastien Petit, Ph.D., *Institut de Chimie des Substances Naturelles, CNRS-ICSN, Université Paris-Saclay, Gif-sur-Yvette, France*
Patrick Carriere Richez, Ph.D., *PIMIT, Processus Infectieux en Milieu Insulaire Tropical, Université de La Réunion, INSERM U1187, CNRS 9192, IRD 249, plateforme CYROI 97490 Sainte-Clotilde, La Réunion, France*
Juliano Haddad, Ph.D., *PIMIT, Processus Infectieux en Milieu Insulaire Tropical, Université de La Réunion, INSERM U1187, CNRS 9192, IRD 249, plateforme CYROI 97490 Sainte-Clotilde, La Réunion, France*
Marc Lemaire, Ph.D., *International Associated Laboratory, University of Antananarivo-Lyon 1, Antananarivo, Madagascar*
Voahangy Vestalys Ramanandraibe, Ph.D., *International Associated Laboratory, University of Antananarivo-Lyon 1, Antananarivo, Madagascar*
Dominique Strasberg, Ph.D., *Unité Mixte de Recherche Peuplements Végétaux et Bio-agresseurs en Milieu Tropical (PVBMT), Pôle de Protection des Plantes, Université de La Réunion, 7 Chemin de l'IRAT, 97410 Saint-Pierre, La Réunion, France*
Cécile Apel, *Institut de Chimie des Substances Naturelles, CNRS-ICSN, Université Paris-Saclay, Gif-sur-Yvette, France., Gif-sur-Yvette, Paris, France*
Marc Litaudon, Ph.D., *Institut de Chimie des Substances Naturelles, CNRS-ICSN, Université Paris-Saclay, Gif-sur-Yvette, France*
Anne Bialecki, Ph.D., *CHEMBIOPRO (Laboratoire de Chimie et de Biotechnologie des Produits Naturels), Faculté des Sciences et Technologies, Université de La Réunion, La Réunion, France*

Mosquito-borne dengue disease has recently evolved into a major public health problem in the South West Indian Ocean (SWIO) region, including Seychelles and Reunion islands, where large epidemics occur since 2016. SWIO islands are classified as a biodiversity hotspot due to its exceptional flora and endemic plants. The goal of our project was to identify endemic plants from Madagascar and Reunion Island active against Dengue (DENV) and Zika (ZIKV). Thus, an in vitro antiviral screening of 430 and 1200 extracts of endemic plants from Reunion Island and Madagascar, respectively, was performed against ZIKV and DENV. This screening yielded several endemic plants, such as Turraea thouarsiana, Turraea ovata, Carissa spinarum, Bertiera borbonica, Trachylobium verrucosum, Comniphora sp, Quivisianthe papinae, that are able to prevent viral infection of Vero cells with a selectivity index greater than 10. Thus, a bio-guided fractionation was undertaken to isolate and characterize active molecules isolated from selected plants. Four tetranortriterpenes compounds belonging to the limonoids group have been isolated from Turrarea thourasiana including a novel structure such as Chisiamol H. Interestingly, four pure compounds belonging to lignan were isolated from Comniphora sp including Justicidin B with an IC50 of 3 µM. Our results highlight the importance of endemic plants from South West Indian Ocean as a promising source of natural antiviral compounds to prevent DENV and ZIKV infection.

213. The Lipid Liaisons of Secreted Dengue Virus NS1 – a Structural Insight

Subhash Vasudevan, Ph.D., *Duke-NUS Medical School, Singapore*
Alvin Bing Liang Chew, Ph.D., *LKC School of Medicine, Nanyang Technological University, Singapore*
An Qi Ngoh, Ph.D. Seeking, *Duke-NUS Medical School, Singapore*
Wint Wint Phoo, Ph.D., *Duke-NUS Medical School, Singapore*
Wing Ki Kitti Chan, Ph.D., *Duke-NUS Medical School, Singapore*
Zheng Ser, Ph.D., IMCB, *A*STAR, Singapore*
Radoslaw Sobota., IMCB, Ph.D., *A*STAR, Singapore*
Dahai Luo, Ph.D., LKC School of Medicine, *Nanyang Technological University, Singapore*

Recent studies have shown that therapeutic antibodies targeted at secreted NS1(sNS1) can prevent endothelial dysfunction that is associated with severe dengue infections. In order to uncover the biologically relevant structure of sNS1, we extracted the native form of sNS1 from cells infected with either the DENV WT or NS1 T164S variant whose appearance was associated with a dengue outbreak in Cuba. We determined the cryoEM structures of sNS1 and its complex with a monoclonal antibody/Fab and found that the major species of sNS1 is a 1:1 complex of the NS1 dimer embedded in a High-Density Lipoprotein (HDL) particle. Cross-linking MS studies confirm NS1:ApoA1 dimer formation with most ApoA1 interaction sites mapped to the NS1 wing and hydrophobic domains. Our results report the molecular architecture of the biological form of NS1 which shed fresh light on the molecular pathogenesis of dengue.

214. Tick-Borne Encephalitis Virus-infected Human Neuronal/glial Cells Identify Antiviral Drugs

Muriel Coulpier, Ph.D., *INRAE, Maisons-Alfort, France*
Noemie Berry, Ph.D., *INRAE, France*
Gaëlle Gonzalez, Ph.D., *ANSES, Maisons-Alfort, France*
Anne Huard de Verneuil, Ph.D., *Pasteur Institute, Paris, France*
Valentine Chaillot, M.S., *INRAE, Maisons-Alfort, France*
Mazigh Fares, Ph.D., *University of GlasGow Center For Virus Research, Glasgow, United Kingdom*
Marielle Cochet-Bernoin, M.S., *INRAE, Maisons-Alfort, France*
François Piumi, M.S., *INRAE, Maisons-Alfort, France*
Kamila Gorna, M.S., *Anses, Maisons-Alfort, France*
Aicha Dembele, M.S., *INRAE, Maisons-Alfort, France*
Peggy Suzanne, M.S., *CERMN, Caen, France*
Odile Blanchet, Ph.D., *CHU Angers, Angers, France*
Hakim Ahmed-Belkacem, Ph.D., *INSERM, Créteil, France*
Patrick Dallemagne, Ph.D., *CERMN, Caen, France*
Nathalie Aulner, Ph.D., *Institut Pasteur, Paris, France*
Bertrand Pain, Ph.D., *INRAE, Lyon, France*
Anne Danckaert, Ph.D., *Institut Pasteur, Paris, France*

Tick-Borne Encephalitis Virus (TBEV), a member of the Flaviviridae family, is the major arbovirus of health interest in Central/Northern Europe and North-Eastern Asia. It is responsible for neurological manifestations that may cause permanent disability or death. There is currently no therapeutic treatment for the disease. Although many studies have been conducted using murine models, there is a lack of relevant in vitro human models for neuropathological studies and drug discovery. Here, we infected human neuronal/glial cells (hNGCs) with TBEV and showed that in vitro infection reproduced major hallmarks of TBEV infection in the human brain, such as preferential neuronal tropism, neuronal death and astrogliosis. We then compared the antiviral activity of 8 selected molecules in hNGCs, human neural progenitor cells (hNPCs) and A549 cell line, as 3 models of TBEV infection of different relevance. We showed that most of the molecules had an antiviral activity in A549 but only one was efficient in hNGCs, demonstrating the importance of physiologically relevant models. Next, we developed an image-based phenotypic screen using hNGCs and tested a hundred and ninety compounds for their ability to restrict viral infection. This led to the identification of new antiviral compounds amongst which two may be therapeutically repositioned as they are drugs currently used in human medicine. The antiviral activity of one of them was further confirmed in a newly developed model of human cerebral organoids infected with TBEV. Such physiologically relevant 2D/3D in vitro models of TBEV infection offer a platform that should accelerate the identification of high value antiviral molecules.

215. Tissue-specific Expansion of Isogenic Variants in the ZIKV Quasispecies Drive Disease Pathogenesis

Kitti W. K. Chan, Ph.D., *Duke-NUS Medical School, Singapore*
Amanda Makha Bifani, Ph.D., *Duke-NUS Medical School, Singapore*
Satoru Watanabe, Ph.D., *Duke-NUS Medical School, Singapore*
Milly M. Choy, Ph.D., *Duke-NUS Medical School, Singapore*
Eng Eong Ooi, Ph.D., *Duke-NUS Medical School, Singapore*
Subhash G. Vasudevan, Ph.D., *Duke-NUS Medical School, Singapore*

The Asian lineage Zika virus (ZIKV) emerged as a public health emergency in 2016 exhibiting severe neuronal pathologies with no apparent historical correlate to the mild disease-causing innocuous member of the mosquito-borne flavivirus genus that was discovered in Africa in 1947. Replication error rate of RNA viruses combined with viral protein/RNA structural plasticity can lead to evolution of virus-induced pathogenicity that is critical to identify and validate for therapeutic interventions. Here we found that quasispecies in the ZIKV French Polynesian clinical strain H/PF/2013 is driving the disease pathogenesis. NextGen Sequencing analysis of the low-passage inoculum virus as well as mouse serum, brain and testis derived virus, revealed specific enrichment of low allele frequency variants in the mouse brain that were not found in the other tissues. Specifically non-structural (NS) protein 2A variant at position 117 along with changes in NS1 and NS4B were uniquely associated with the mouse brain isolate. Mutational analysis of these variants in cDNA infectious clone demonstrated that NS2A as the lethal pathogenic determinant with potential epistatic contribution of NS1 and NS4B variants in ZIKV brain pentrance. Together our study highlights the importance of examining the low-lying variants within the virus swarm in disease pathogenesis, which could serve as predictors of severe epidemics as well as providing insights into potential viral targets for antiviral development.

216. Trehalose Monolaurate Suppresses Dengue Virus Infection Through Multiple Mechanisms

Yi-Jung Ho, Ph.D., *National Defense Medical Center, Taipei, Taiwan, Republic of China*
Jeng-Wei Lu, Ph.D., *Singapore-MIT Alliance for Research and Technology (SMART), Taiwan, Republic of China*
Yen-Chen Chen, Pharm.D., *National Defense Medical Center, Taipei, Taiwan, Republic of China*

Dengue disease is the most common mosquito borne disease in the worldwide. Half of population live in the threat of dengue virus (DENV) infection. However, it still lacks the antiviral for clinic use. Trehalose monolaurate (TML) is a sugar ester which is considered with good biodegradability and less toxicity. In pharmaceutical, TML could be used as permeation enhancers or protein stabilizer and also could be used to establish drug delivery system. However, there is no any evidence about the antiviral ability of TML. Herein, we proved that TML might possess the antiviral ability in dengue virus (DENV)infection. TML inhibited the viral RNA level, protein production and viral yield of DENV in a dose-dependent manner. In the data of time of addition assay, TML had significant inhibition of DENV infection at the co-treatment stage and post-treatment stage. Furthermore, TML also revealed its inhibition at binding assay, entry assay, replication assay and virus release assay. TML also influenced the infectivity of DENV by disrupting virion stability. The results indicated that TML might through various mechanism to suppress DENV infection. Finally, we had also proved the anti-viral ability of TML in those serotypes of DENVs. In summary, TML could inhibit DENV infection through multiple mechanisms and might possess broad antiviral ability of different serotypes of DENVs infection.

217. Trehalose-based Esters Possess an Antiviral Ability toward Sindbis Virus

Yen-Chen Chen, Pharm.D., *Institute of Preventive Medicine, Taipei, Taiwan, Republic of China*
Yi-Jung Ho, Ph.D., *National Defense Medical Center, Taiwan, Republic of China*
Jeng-Wei Lu, Ph.D., *Singapore-MIT Alliance for Research and Technology (SMART), Taiwan, Republic of China*

Alphaviruses belong to Togaviridae, which includes Chikungunya virus (CHIKV), Semliki Forest virus (SFV), and Sindbis virus (SINV). Ockelbo disease in Sweden, Pogosta disease in Finland, and Karelian fever in Russia all result from SINV infection. The clinical symptoms include fever, rash, and arthralgia, but some cases would suffer from SINV-induced joint symptoms for several months like CHIKV infection. Unfortunately, there are not any approved antivirals for SINV infection. Trehalose-based esters, such as trehalose monolaurate (TML) and trehalose monooleate (TMO), are biodegradable and have less toxicity. They were used to enhance the absorbance of oral formulation or to stabilize protein formulation. In this study, we found that both TML and TMO could inhibit SINV infection, determined by immunofluorescence assay, and the IC50 values of TML and TMO were 83.75μM and 65.07μM respectively. The CC50 values of TML and TMO were >200 μM and > 100μM. Besides, TML was also proved to be able to effectively

decrease viral RNA expression, protein production, and viral yield. The time of addition assay indicated that TML mainly acted at the late stage of viral infection. Viral replication assay and release assay further showed that TML significantly decreased the viral RNA level and virion release in a dose-dependent manner. The transcriptome profiles of SINV-infected U2OS cells with or without TML treatment were analyzed to find out the cellular pathways altered by TML. Finally, TML also was proved a significant inhibition of CHIKV infection. Our finding indicated that TML could inhibit SINV infection after virus entry and might possess a broad antiviral effect in alphaviruses.

218. Viral RNA Decapping Enzyme Chikungunya Virus nsP1 Activates Type I Interferon Signalling Pathway

Michelle Law, Ph.D. Seeking, *Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore*
Dahai Luo, Ph.D., *Nanyang Technological University, Singapore*
Kuo Zhang, Ph.D., *Singapore*
Trinh Mai Nguyen, B.S., *Singapore*
Yaw Bia Tan, Ph.D. Seeking, *Singapore*
Yee Song Law, Ph.D., *Singapore*
Melissa Wirawan, Ph.D., *Singapore*

Alphavirus nsP1 is a dodecameric ring structure where each unit contains both the N7-guanine methyltransferase and guanylyltranferase activities which are key steps in the capping of viral mRNA. We demonstrate the functional versatility of CHIKV nsP1 through the reverse catalytic process, as a novel decapping mechanism the virus may use to employ for continued replication and transcription. CHIKV nsP1 can decap a variety of RNA substrates including host mRNAs, triggering RIG-I mediated IFN response, this could be a new mechanism through which alphaviruses activate the antiviral immune response. This function, we also determined high-resolution cryo-EM structures of Chikungunya virus (CHIKV) nsP1 in complex with short capped RNAs such as m7GpppAmU demonstrating decapping in vitro. We also compare its structure to other known decapping enzymes such as DcpS and L-A virus Gag protein. Future antivirals that interfere with nsP1 capping and decapping activity have downstream implications in the host immune response.

219V. A Yellow Fever Virus NS4B Inhibitor Executes Multiple Mode-of-Action

Fuxuan Wang, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Zhao Gao, *United States*
Lin Zhang, *United States*
Sumangala Darsandhari, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Lauren Griffith, *United States*
Julia Ma, *United States*
Abbie E. Weight, B.S., *Utah State University, Logan, UT, United States*
Justin Julander, Ph.D., *Utah State University, Logan, UT, United States*
Yanming Du, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Ju-Tao Guo, M.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

We previously reported a yellow fever virus (YFV) NS4B inhibitor BDAA with potent in vivo and in vitro efficacy. Our data supported a model that the interaction of BDAA with YFV NS4B impairs the integrity of YFV replication organelles (ROs), which not only directly abrogates viral replication, but also promotes viral RNA release from RO to activate cytosolic dsRNA sensors. Recently, we found that BDAA treatment accelerates typical apoptosis and caspase-dependent secondary pyroptosis but not necroptosis in YFV infected cells. In addition, genetic evidence demonstrated that the BDAA-enhanced cell death depends on the activation of multiple cytosolic dsRNA sensing pathways, including RLR-MAVS, PKR and OAS-RNase L. Interestingly, the antiviral potency of BDAA was not apparently affected in the cell line with all three cytosolic RNA sensing pathway key components knocked out, suggesting that the primary antiviral action of BDAA can be independent of the dsRNA-mediated immune activation. Indeed, BDAA directly inhibits nascent YFV RNA synthesis in cell culture using a 5-ethynyl uridine incorporation assay as well as an endogenous polymerase reaction, within 30 mins to 1 hr of treatment. Taken together, our new data further support that BDAA primarily hits the YFV RO and executes multiple mode-of-action (MOA). Although the MOA involves activation of innate immune response and premature killing of infected cells, the primary antiviral action of the YFV NS4B inhibitors is direct inhibition of viral RNA replication. The MOA of BDAA and its analogs is currently under investigation in hamster model to support ongoing preclinical development of this family of YFV NS4B inhibitors.

220V. Trans-dominant Inhibition of DENV Replication Mediated by a NS3 Internal Cleavage Site

Milly Choy, Ph.D., *Duke-NUS Medical School, Singapore*
Wing Ki Kitti Chan, Ph.D., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*
Eunji Hong, *Duke-NUS Medical School, Singapore*
Crystall Swarbrick, *Griffith University, Australia*
Gerasimos Rassias, *University of Patras, Patra, Greece*
Subhash Vasudevan, Ph.D., *Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore*

Dengue poses a significant global health burden. The dengue virus genome is translated into a single polyprotein and correct processing of the polyprotein by both host and viral proteases is critical for efficient viral replication. The viral polyprotein gets processed at the lumen side of the rough endoplasmic reticulum membrane by the host cell peptidase whereas dengue NS3 serine protease and its cofactor NS2B cleaves the polyprotein at the cytoplasmic side. In addition to processing at junctions between adjacent NS proteins, the NS2B/3 protease also mediates cis-cleavage of internal sites within NS3. These internal cleavage sites are highly conserved across members of the Flaviviridae family, although the role they play in the virus life cycle remains unclear. We have previously postulated that a potent antiviral amidoxime prodrug positions itself between the protease and helicase domains of NS3 to occlude the internal cleavage site, thus presenting NS3 internal cleavage at this site as a potential antiviral target. Mutational analysis of the internal cleavage site positions revealed that G459L mutation in NS3 inhibits self-cleavage of NS3 and inhibits viable virus production. Interestingly, presence of this mutant viral genome and not RNA genomes carrying mutations in other key residues in NS3 or NS5 (eg. NS3 R458Q, N570A, NS5 R330A or NS5 "GDD" to 'GAA") suppresses wild-type virus replication in a phenomenon called trans-dominant inhibition. Exploring this internal cleavage site pocket and elucidating the mechanism of the trans-dominant inhibition of the NS3 internal cleavage site will improve current antiviral drug design that extend beyond conventional treatment.

300. A Lipopeptide Fusion-inhibitor Platform for Preventing and Treating Enveloped Viral Infection

Matteo Porotto, Ph.D., *Columbia University Vagelos College of Physicians & Surgeons, New York, NY, United States*
Ariel Kuhn, *United States*
Anirban Das, *United States*
Francesca Bovier, *United States*
Tara Marcink, *United States*
Victor Outlaw, *United States*
Camilla Predella, *Department of Pediatrics, Columbia University Medical Center, New York, United States*
Cyrille Mathieu, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, Rhone, France*
Shari Stenglein, *United States*
Daniella Iezza, *United States*
Allen Duplantier, *Thylacine Biotherapeutics Inc, United States*
Joan Fusco, *Thylacine Biotherapeutics Inc, United States*
Valerio Dorrello, *United States*
Barry Rockx, *Netherlands*
Stefan Niewiesk, *United States*
Branka Horvat, M.D., Ph.D., *CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS, UMR5308, Univ Lyon, Université Claude Bernard Lyon 1, École Normale Supérieure de Lyon, Lyon, Rhone, France*
Rory de Vries, *Netherlands*
Rik de Swart, *Netherlands*
Christopher A. Alabi, Ph.D., *Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, United States*
Samuel Gellman, Ph.D., *University of Wisconsin - Madison, United States*

For many enveloped viruses, infection is initiated by fusion of the viral and host cell membranes, mediated by a viral fusion protein or protein complex. We have developed broad spectrum fusion inhibitors that prevent viral infection and/or transmission. Upon viral attachment and/or uptake, conformational rearrangements occur in the fusion protein, driven by formation of a structure that couples protein refolding directly to membrane fusion. Fusion inhibitory peptides

target the formation of this structure and block fusion. Structure-based optimization of fusion inhibitory lipopeptides was combined with backbone modification to enhance peptide half-life and lipid conjugation to enhance antiviral efficacy. We validated the lipopeptide platform with experiments demonstrating antiviral efficacy in vitro and in vivo for paramyxoviruses that fuse at the cell surface (e.g. measles, parainfluenza, and Nipah viruses) or in the endosome (e.g. influenza and Ebola viruses), and for SARS-CoV and MERS viruses and multiple SARS-CoV-2 virus variants. For SARS-CoV-2, daily intranasal administration to ferrets completely prevented direct-contact transmission during 24-hour co-housing with infected animals, under stringent conditions that resulted in infection of all untreated animals. The same lipopeptide is effective against SARS-1, MERS, and multiple emerging variant coronaviruses in vitro and is currently under translational development for in vivo pancoronavirus efficacy via inhaled administration. The lipopeptide platform enables rapid design and scale-up for prophylaxis or early treatment and offers an approach to block transmission in the face of emerging enveloped viruses.

301. A Systematic Study of SARS-CoV-2 Main Protease Drug Resistant Mutants Against Nirmatrelvir and Ensitrelvir

Jun Wang, Ph.D., *Rutgers University: Rutgers The State University of New Jersey, Piscataway, New Jersey, United States*
Yanmei Hu, *Rutgers University: Rutgers The State University of New Jersey, Piscataway, New Jersey, United States*
Haozhou Tan, *Rutgers University: Rutgers The State University of New Jersey, Piscataway, New Jersey, United States*

The SARS-CoV-2 main protease (Mpro) is the drug target of Pfizer's oral drug nirmatrelvir. The emergence of SARS-CoV-2 variants with mutations in Mpro raised the alarm of potential drug resistance. To identify potential clinically relevant drug resistant mutants, we systematically characterized 102 naturally occurring Mpro mutants located at 12 residues at the nirmatrelvir binding site, among which 21 mutations in 5 residues, including S144M/F/A/G/Y, M165T, E166G/A, H172Q/F, and Q192T/S/L/A/I/P/H/V/W/C/F, showed comparable enzymatic activity to the wild-type (kcat/Km <10-fold change) while being resistance to nirmatrelvir (Ki >10-fold increase). We also determined the cross resistance of these high profile Mpro mutants against the clinical candidate ensiltrelvir. X-ray crystal structures were determined for several representative mutants with and/or without GC-376/nirmatrelvir, providing a structural basis for the drug resistance and a guidance for the design of next generation Mpro inhibitors. Using recombinant SARS-CoV-2 viruses generated from reverse genetics, we confirmed the drug resistance in the antiviral assay and showed that Mpro mutants with reduced enzymatic activity had attenuated viral replication. Overall, our study identified several drug resistant hot spots in Mpro that warrant close monitoring for possible clinical evidence of nirmatrelvir resistance. Our study represents one of the most comprehensive characterization of SARS-CoV-2 Mpro mutants, and our genome sequence mining approach identified several mutants that were also selected from the serial viral passage experiment, validating the clinical relevance of our approach.

302. Advanced Junín Mammarenavirus Infection and Disease Effectively Treated with the Broadly Active Ribonucleoside Analog, EIDD-2749

Jonna Westover, Ph.D., *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Alexander A. Kolykhalov, Ph.D., *Emory Institute for Drug Development, Emory University, GA, United States*
Kevin Bailey, B.S., *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, U tah State University, Logan, UT, United States*
Luci Wandersee, B.S., *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Gregory Bluemling, Ph.D., *Emory Institute for Drug Development, Emory University, Atlanta, GA, United States*
Kirsten Boardman, B.S., *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Samantha Wasson, Ph.D. Seeking, *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Gabrielle Rock, B.S., *Department of Animal, Dairy and Veterinary Sciences, Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Shuli Mao, Ph.D., *Emory Institute for Drug Development, Emory University, Atlanta, GA, United States*
Kie Hoon Jung, Ph.D., *Utah State University employee, Logan, Utah, United States*
Michael G. Natchus, Ph.D., *Emory Institute for Drug Development, Emory University, Atlanta, GA, United States*

George R. Painter, Ph.D., *Emory Institute for Drug Development, United States*
Brian Gowen, Ph.D., *Utah State University, Logan, United States*

The World Health Organization considers several mammarenaviruses as significant threats to public health. EIDD-2749 is a broadly active uridine ribonucleoside analog under development as a treatment for viral hemorrhagic fever caused by New and Old World mammarenavirus infections. In proof-of-concept studies, EIDD-2749 treatments were highly effective at controlling New World mammarenavirus infections in mouse and guinea pig disease models. Here, we present advanced EIDD-2749 therapeutic efficacy studies in the Junín virus (JUNV) guinea pig model of Argentine hemorrhagic fever. Remarkably, oral sub-mg/kg doses of EIDD-2749 administered every other day (Q2D) starting one week after JUNV challenge reduced or eliminated viremia and tissue viral loads and protected against severe disease. Even when initiating EIDD-2749 (1.5 mg/kg, Q2D) treatment ten days post JUNV exposure, the compound reversed clinical signs of disease and significantly ($p < 0.01$) improved survival outcomes. In addition to the in vivo studies, efforts to select for JUNV resistance to EIDD-2749 in cell culture by dose-escalation and fixed-dose passage strategies are proving that there is a high genetic barrier to resistance. Overall, the data support the continued development of EIDD-2749 as a promising orally bioavailable small-molecule therapeutic for treating severe disease caused by highly pathogenic mammarenaviruses.

303. Analytical Methods Development for the Measurement of Physico-chemical Parameters of a New Antiviral in Pre-clinical Phase

Luigi Agrofoglio, Ph.D., *ICOA UMR CNRS 7311, Orléans, France*
Thomas Mathieu, *ICOA UMR CNRS 7311, Orléans, France*
Patrick Favetta, *ICOA UMR CNRS 7311, Orléans, France*

Acyclonucleoside phosphonates (ANP) prodrugs, such as Hepsera© (bis(POM)PMEA or Adefovir®dipivoxil) and Viread© (bis(POC)PMPA or Tenofovir® disoproxil), are active against various DNA, RNA and retroviruses. Our group has developed a new family of ANPs based on a trans-but-2-enyl phosphonate moiety. Among the many compounds synthesized, LAVR-289, a triple prodrug, has shown remarkable antiviral properties at nM concentrations on several DNA viruses including POX viruses. Additionally, LAVR-289 has not shown toxicity in mice. At the pre-clinical development stage the physico-chemical parameters such as pKa value, logP, and critical micellar concentration (self-organisation), of LAVR-289 have been determined by UV spectroscopy, HPLC-UV method, and fluorescence spectroscopy, respectively. A liquid chromatography method has been developed to separate isomers Z and E of LAVR-289, because only the Z form is bioactive. This methodology was used in semi-preparative chromatography to purify active molecule in laboratory scale. In the same manner, due to the presence of a chiral phosphorous, the challenging chiral separation of four diastereomers was achieved. We have monitored the chemical and plasma stabilities by HPLC-UV, which allowed us to determine some metabolites (characterized by NMR and HRMS) and find the human plasma half-time stability of around 6 hours. Analytical method will be presented and discussed.

304. Synthesis of Heterocyles Targeting STING Protein in Innate Immunity

Luigi Agrofoglio, Ph.D., *ICOA UMR CNRS 7311, Orléans, France*
Jeremy Magand, *France*
Vincent Roy, Ph.D., *ICAO, Orléans, France*
Stephanie Rose, *France*
Valérie Quesniaux, *France*

STING protein is the most important adaptor protein in immune response. Herein we report on the synthesis of small libraries of hitherto unknown new heterocycles bearing either an oxazolo[5,4-d]pyrimidine and a benzimidazole moiety, respectively. Oxazolo[5,4-d]pyrimidine analogs were obtained from 5-aminouracilthrough an initial arylamide formation followed by a one-pot cyclisation and substitution in POCl. Palladium(0)-catalyzed Suzuki and Stille reactions and SNAr created diversity. Substituted piperazine benzimidazoleanalogues were obtained by a convergent approach based on the coupling of various piperazines to the activated primary alcohol of benzimidazoles, respectively. All final compounds were evaluated (1) in silico on STING protein crystal structure through docking studies, (2) in cellulo by measuring the secretion level of the interferon-induced chemokine CXCL10/IP-10. Detailed synthesis and biological data will be presented. (This work was done with financial support of FEDER EuroFérI and MESRI).

305. Application of Virtual Screening Methods for the Identification of Novel Ebola Virus (EBOV) VP24 Inhibitors

Alessia Onali, *University of Cagliari, Cagliari, Italy*
Angela Corona, Ph.D., *University of Cagliari, Italy*
Annalaura Paulis, M.S., *Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*
Erica Sanna, *University of Cagliari, Cagliari, Italy*
Daniela Secci, *University of Cagliari, Cagliari, Italy*
Rita Meleddu, Ph.D., *University of Cagliari, Cagliari, Italy*
Enzo Tramontano, Ph.D., *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Sante Scognamiglio, *University of Cagliari, Cagliari, Italy*
NicolaAlberto Marcia, *University of Cagliari, Cagliari, Italy*
Elias Maccioni, Ph.D., *University of Cagliari, Cagliari, Italy*
Simona Distinto, Ph.D., *CADD unit Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*

The Ebola virus VP24 is one of the seven proteins encoded by the Ebola virus, and plays an important role in the virus's life cycle. In particular, VP24 is essential for the formation of the virus's outer envelope, the virus's replication and assembly, and it is also involved in the virus's pathogenesis. VP24 can impair cellular antiviral defense by suppressing both IFN-α/β and IFN-γ signaling and, moreover, it acts by binding the karyopherin-α (KPNα), preventing its interaction with phosphorylated STAT1, essential for activate transcription of IFN-stimulated genes. These features make it an attractive target for drug development. Both ligand and structure-based strategies have been exploited to identify potential inhibitors of Ebola-VP24. A total of 17 compounds were selected and subject to biological tests.

306. Approaches to Develop icDNA Clones of Encephalitic Alphaviruses to Characterize Viral Disease and Evaluate Broad-spectrum Therapeutics

Rachel Ireland, Ph.D., *DSTL, Salisbury, United Kingdom*
Amanda Phelps, *DSTL, Salisbury, United Kingdom*
Michelle Nelson, Ph.D., *DSTL, Salisbury, United Kingdom*

The Equine Encephalitis alphaviruses pose a significant risk to both animal and human health due to their potential for high morbidity and/or mortality and there are no licensed vaccines or antivirals to treat these infections. There is limited clinical data relating to alphavirus pathogenesis and many of the existing animal models are unable to recapitulate the encephalitic disease often arising from infection. Of particular concern, is the increased potential for neuroinvasion following aerosol infection, which may result in an increased incidence of encephalitis in humans, and even death. To this end, the aim of this study was to develop an approach to synthesize infectious cDNA (icDNA) clones of Equine Encephalitis alphaviruses that could be genetically modified to incorporate light-emitting reporters. These reporter viruses could be used to study alphavirus pathogenesis and neuroinvasion in animal models, and furthermore used to evaluate the efficacy of vaccines and broad-spectrum antivirals. A proof-of-concept study has been conducted to generate an icDNA clone of wild-type Eastern Equine Encephalitis virus (EEEV) and characterise this clone for replicase activity, in vitro growth and aerostability. In addition, the virulence of the icDNA clone of EEEV was confirmed in an inhalational Balb/c mouse model where mice developed key features of disease such as acute onset of neurological signs with high titres of virus isolated from the brain. Further studies are in progress to genetically modify the wild-type icDNA clone to incorporate fluorescent/bioluminescent reporters to allow in vivo imaging of EEEV in real-time.

307. Assay Development for High-throughput Antiviral Compound Screening Against Bunyavirus L Protein

Dominik Vogel, Ph.D., *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
Janna Scherf, M.S., *Bernhard Nocht Institute for Tropical Medicine, Germany*
Maria Rosenthal, Ph.D., *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
Carola Busch, *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
Jeanette Reinshagen, *Fraunhofer Institute for Translational Medicine and Pharmacology ITMP Hamburg, Hamburg, Germany*
Sheraz Gul, Ph.D., *Fraunhofer Institute for Translational Medicine and Pharmacology, Hamburg, Germany*
Philip Gribbon, Ph.D., *Fraunhofer Institute for Translational Medicine and Pharmacology, Hamburg, Germany*

The order of Bunyavirales is a highly diverse group including severe human pathogens. The WHO R&D Blueprint emphasizes the urgent need for medical countermeasures against several bunyaviruses, including the Lassa and the Rift Valley fever virus which are a threat to global health and the economy especially in low- and middle-income countries. Most of these viruses must be handled in biocontainments of the highest biosafety standards limiting the method and technology spectrum for research. Bunyaviruses are segmented negative-strand RNA viruses. The termini of each genome segment serve as regulatory elements essential for genome replication and transcription. Both processes are catalyzed by the viral L protein (L), which contains an RNA-dependent RNA polymerase (RdRp), an endonuclease (EN) and a cap-binding domain (CBD). While viral genome replication is initiated de novo, for transcription bunyaviruses use a cap-snatching mechanism. This involves the CBD and EN acting to bind and cleave off capped fragments of host mRNA which are subsequently used to prime viral transcription. To identify potential antiviral compounds, we seek to validate the multiple functions of the bunyavirus L as targets for inhibitors. To this end we develop fluorescence-based assays using recombinant full-length L protein. We were able to establish robust and reliable miniaturized fluorescence polarization and homogeneous time-resolved fluorescence assays with high sensitivity applicable for high-throughput screening of small molecule compound libraries. The established in vitro assays allow for compound screening without the need for high biosafety containments.

308. Broad-spectrum Antiviral Activity of Polyoxometalates

Manuela Donalisio, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*
Irene Arduino, Ph.D. Seeking, *Department of Clinical and Biological Sciences, University of Turin, Torino, Italy*
Roberta Cavalli, Ph.D., *Department of Drug Science and Technology, University of Turin, Torino, Italy*
Ulrich Kortz, Ph.D., *Department of Life Sciences and Chemistry, Jacobs University, Bremen, Germany*
David Lembo, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*

Polyoxometalates (POMs), anionic metaloxo complexes of early transitional metal ions in high oxidation states, have emerged in last years as potentially useful agents in medicine for their biological properties, including the antiviral activity. Here we report on the screening of a minilibrary of POMs, which were all synthesized previously, against a panel of DNA and RNA viruses on cell cultures, including herpetic viruses, adenovirus, papillomavirus, vaccinia virus, rotavirus, zikavirus, vesicular stomatitis virus, respiratory syncytial virus, rhinovirus and coronaviruses. Interestingly, we identified a Keggin-type POM (Ti2PW10), endowed with broad-spectrum inhibitory activity against all viruses tested except for rotavirus and free of cytotoxicity over a wide range of doses tested. Further studies were performed to assess its antiviral potential against herpetic viruses and respiratory viruses given its highly favorable selectivity indexes (SIs >700). Mechanism of action studies demonstrated that the selected POM does not inactivate viral particles, but it affects the early steps of the viral replicative cycle, hampering the viral entry into host cells. No inhibitory activity was observed treating cells before the infection. Of note, no viral resistant variants were selected by the serial passage approaches using rhinovirus A1 and coronavirus HCoV-OC43, two RNA viruses, known to constantly evolve through mutations. Further work needs to be carried out to identify the cellular or viral molecular targets involved in the inhibition of viral infections. This work poses POMs as promising candidates to develop first-line broad spectrum therapeutics to contrast viral diseases.

309. Combinations of Host- and Virus-targeting Antiviral Drugs Confer Synergistic Suppression of SARS-CoV-2

Stephen Polyak, Ph.D., *University of Washington, Seattle, WA, United States*
Jessica Wagoner, *University of Washington, Seattle, WA, United States*
Shuang Xu, *Fred Hutchinson Cancer Research Center, United States*
Judith White, *University of Virgina, United States*
Joshua Schiffer, *Fred Hutchinson Cancer Research Center, United States*

It took over two years to develop oral antiviral drugs for SARS-CoV-2 and current options are limited to two drugs, molnupirivir (MPV) and paxlovid (PAX). Since combination antiviral drug treatments are the norm for chronic RNA virus infections, and since our past work showed that combinations of approved drugs can synergistically inhibit filoviruses (e.g., Ebola) and arenaviruses (e.g., Lassa), we continue to advance the development of self-administered drug cocktails as a first line of defense against viral pathogens. Here, we demonstrate that combining host-targeting antivirals (HTA) that target TMPRSS2 and hence SARS-CoV-2 entry, with MPV, a virus-targeting directly acting antiviral (DAA) that targets SARS-CoV-2 replication, synergistically suppresses SARS-CoV-2 infection in Calu-3 lung epithelial cells. Strong

synergy was observed when MPV was combined with three TMPRSS2 oral or inhaled inhibitors: camostat, avoralstat, or nafamostat. The combination of camostat + MPV was also effective against the beta and delta variants of concern (VOC). The pyrimidine biosynthesis inhibitor brequinar combined with MPV also conferred robust synergistic inhibition. These HTA+DAA combinations had similar potency to the synergistic all-DAA combination of MPV + nirmatrelvir, the protease inhibitor found in PAX. Pharmacodynamic modeling allowed estimates of antiviral potency at all possible concentrations of each agent within plausible therapeutic ranges, suggesting possible in vivo efficacy. The triple combination of camostat, brequinar, and MPV further increased antiviral potency. These findings support development of HTA and DAA combinations for pandemic response and preparedness.

310. Crystal Structure of the 2′-O-ribose Methyltransferase VP39 from Monkeypox Virus in Complex with Sinefungin

Jan Silhan, Ph.D., *Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic*
Klima Martin, Ph.D., *Institute of Organic Chemistry and Biochemstry, Czech Republic*
Petr Skvara, M.S., *Institute of Organic Chemistry and Biochemstry, Prague, Czech Republic*
Dominika Chalupska, Ph.D., *Institute of Organic Chemistry and Biochemstry, Prague, Czech Republic*
Tomas Otava, M.S., *Institute of Organic Chemistry and Biochemstry, Prague, Czech Republic*
Jan Kozic, *Institute of Organic Chemistry and Biochemstry, Prague, Czech Republic*
Radim Nencka, Ph.D., *Institute of Organic Chemistry and Biochemstry, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*

The Poxviridae family of viruses includes smallpox and vaccinia viruses and its genome is encoded by dsDNA and replicates in the cytoplasm of infected cells. In May 2022, the outbreak caused by the emerging monkeypox virus (MPXV) spreading around the world is a new pandemic threat. The monkeypox genome encodes its own RNA processing machinery, including the capping machinery for producing its own mRNA and proteins. In this work, we produced the recombinant 2′-O-RNA methyltransferase (MTase) VP39 and crystallized it in complex with the pan-MTase inhibitor sinefungin. Small plate-like crystals diffracted to a resolution of 2Å and allowed us to solve the crystal structure. A comparison of this 2′-O-RNA MTase with enzymes from unrelated ssRNA viruses (SARS-CoV-2 and Zika) revealed a surprisingly conserved sinefungin binding mode, implying that a single inhibitor could be used against unrelated virus families. We tested several of these compounds to biochemically validate this observation and uncover their true potential.

311. Deep Sequencing Reveals Correlates of Prophylactic Protection of BDGR-49 in Mice Intranasally Challenges with Venezuelan Equine Encephalitis Virus Trinidad Donkey

Evan Williams, Ph.D., *Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, United States*
Jyothi Parvathareddy, B.S., *Regional Biocontainment Laboratory, University of Tennessee Health Science Center, Tennessee, United States*
Dong Yang, Ph.D., *Regional Biocontainment Laboratory, University of Tennessee Health Science Center, Memphis, Tennessee, United States*
Xufeng Cao, Ph.D., *Department of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States*
Jennifer Golden, Ph.D., *Department of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, Madison, Wisconsin, United States*
Colleen Jonsson, Ph.D., *Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, Tennessee, United States*

Venezuelan Equine Encephalitis Virus (VEEV), a new world Alphavirus, causes a febrile illness that may result in fatal neurological diseases in humans and equids. Currently, there are no FDA-approved antivirals for the treatment of human infection of this virus. To combat this, we developed a novel brain-penetrant, small molecule, BDGR-49, which when administered subcutaneously at 6 mg/kg twice per day for 6 days conferred 100% protection against a lethal intranasal challenge of VEEV Trinidad donkey in BALB/c mouse model. By 8 days post-infection (dpi), viral load in the brain was significantly reduced whereas infected and mock-treated mice succumbed to disease on 5 dpi. Transcriptional

analyses of the brains of infected and treated or mock-treated mice corroborated infectious virus titers. Analysis of the host responses in the brains showed treatment resulted in a significant reduction in expression of genes in pathways associated with hypercytokinemia, macrophage signaling, phagosome formation, and S100 signaling. With this, BDGR-49 confers complete protection in mice with a reduction of both infectious and viral RNA levels as well as a reduction in activation of host response.

312. Design, Synthesis, and Biological Evaluation of Expanded (Linker) Flex-Acyclovir Analogues as Potential Broad-Spectrum Antiviral Drugs

Joy E. Thames, Ph.D. Seeking, *University of Maryland, Baltimore County, Baltimore, MD, United States*
Kyle A. Davis, B.S. Seeking, *University of Maryland, Baltimore County, MD, United States*
Katherine L. Seley-Radtke, Ph.D., *University of Maryland, Baltimore County, Baltimore, MD, United States*

As the SARS-CoV-2 pandemic continues to affect people worldwide, it is clear there is a need for broad-spectrum antiviral therapeutics to not only help fight the pandemic at hand but help fight future pandemics to come. With this in mind nucleos(t)ides have had a rich history as antivirals. A commonly employed modification is the use of an acyclic sugar, such as that found in Acyclovir (ACV), an FDA-approved drug for herpes simplex virus. Research in the Seley-Radtke group focuses on investigating nucleos(t)ide analogues known as "fleximers", which feature a purine nucleobase "split" into its imidazole and pyrimidine moieties via a carbon-carbon single bond, thus introducing flexibility to the nucleobase. This novel design has endowed the fleximer analogues with potent activity not seen in the rigid parent nucleosides. Combining the acyclic sugar, with the fleximer technology produced a series of doubly flexible Flex-ACV analogues. Some of these analogues have exhibited activity against filoviruses, flaviviruses, and coronaviruses. A new series of Flex-ACV analogues that feature "linkers" have been synthesized. The expanded fleximers are of interest because they can either be more flexible or alternatively, more rigid, depending on the group inserted between the nucleobase moieties. For example, insertion of an ethynyl group leads to rigidity, while the addition of an -NH- or -CH2- allows for more rotation/flexibility. Computational modeling of the expanded Flex-ACV analogues showed promising results in the Dengue 3-NS5 binding site. The results of those studies, with the synthesis and biological activity of the Flex-ACV analogues will be reported herein.

313. Development of a Cell-Based Assay for Evaluating b-coronavirus Inhibitors

Rebekah J. Dickmander, *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*
Erik Lenarcic, *University of North Carolina at Chapel Hill, NC, United States*
Marcia Sanders, *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*
Sharon Taft-Benz, *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*
Mark Heise, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*
Nathaniel Moorman, Ph.D., *University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

The SARS-CoV-2 pandemic has highlighted the need to discover and develop broad-spectrum antiviral drugs to prepare for the next pandemic. A major barrier to these efforts is the need to work in high containment lab settings (e.g. BSL3 labs). To address this need, we developed an approach to evaluate anti-coronavirus compounds using a recombinant mouse hepatitis virus, a b-coronavirus closely related to SARS-CoV-2, engineered to express nanoluciferase (MHV-nLuc) from the viral genome. We confirmed that nLuc levels in infected cells directly correlate to levels of virus replication, and found that the assay was highly reproducible with a wide dynamic range. The assay is rapid, compatible with high throughput screening approaches, and can be used in BSL2 laboratories. We tested over 1,000 compounds including known SARS-CoV-2 inhibitors for antiviral activity and found that activity against MHV-nLuc was predictive of antiviral efficacy against SARS-CoV-2. In addition to screening, we have successfully used this assay to guide structure-activity relationship campaigns to optimize the antiviral efficacy of small molecules targeting the human CSNK2 and PIKfyve kinases, as well as direct acting antiviral inhibitors. This assay provides a rapid and reproducible assay to enable researchers without access to high containment labs to engage in coronavirus antiviral drug discovery and development.

314. Development of a Virucidal Compound Against Influenza Viruses

Caroline Tapparel, *Université de Genève, Genève, Switzerland*
Chiara Medaglia, *Switzerland*
Arnaud Zwygart, *Université de Genève, Geneva, Switzerland*
Jacob Silva Paulo Henrique, *EPFL, Lausanne, Switzerland*
Valeria Cagno, *CHUV, Lausanne, Switzerland*
Francesco Stellacci, *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Clément Fage, Ph.D., *Université de Genève, Geneva, Switzerland*

Influenza viruses (IVs) are major public health concerns, with an annual death toll of more than 500,000 people worldwide. Due to their error-prone polymerase, IVs rapidly acquire genetic variability that hinders vaccine effectiveness and inevitably culminates in the emergence of resistance to antivirals. For this reason, the development of new antiviral therapeutic compounds targeting conserved viral domains and displaying a high genetic barrier to antiviral resistance, should be a research priority. In our study, we developed, in collaboration with Prof. Stellacci (EPFL), an antiviral compound, SA11-CD, mimicking sialic acid (SA), the cellular receptor of IVs. In vitro experiments demonstrated that SA11-CD (1) is able to bind the HA of several IVs strains (type A and B) and inhibits their replication; (2) displays selective virucidal property being able to irreversibly inactivate influenza A/H1N1 and influenza B (Yamagata lineage) viruses but not H3N2 nor H5N1 viruses. Nevertheless, our study also highlighted that resistance against SA11-CD emerged in vitro. In order to prevent it, we combined SA11-CD with IFN λ and demonstrated that it successfully delayed emergence of resistance against SA11-CD. Finally, we confirmed the efficacy of SA11-CD in vivo, using a mouse/H1N1 influenza infection model. After intra-nasal treatments, our compound did not show toxicity and demonstrated high efficacy in protecting mice from H1N1 infection when administered 24h or 48h post infection. In conclusion, our study highlights the high therapeutic potential of SA11-CD and the need to improve our strategy to produce new antiviral compounds with high barrier to antiviral resistance.

315. Development of Molecularly Defined Broad-spectrum Virucidal Drugs

Yong Zhu, Ph.D. Seeking, *Institute of Materials Science & Engineering, Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Matteo Gasbarri, Ph.D., *Institute of Materials Science & Engineering, Institute of Bioengineering, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
GregoryTristan Mathez, Ph.D. Seeking, *Institute of Microbiology, Lausanne University Hospital, University of Lausanne, Lausanne, Waadt, Switzerland*
Valeria Cagno, Ph.D., *CHUV, Lausanne, Switzerland*
Francesco Stellacci, *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

Viruses enter host cells by binding to cell-surface macromolecules, such as heparan sulfate. Antivirals that mimic these macromolecules block viral attachment by competitively binding to viral proteins. A multivalent scaffold is typically required to improve the binding affinity. Most studies focused on exploring the optimal ligand density on the scaffold, while the scaffold internal structure, such as hydrophobicity and charge pattern, remains less investigated. In this study, we synthesized a library of antivirals featuring various small-molecule cores modified with multivalent heparan sulfate-mimicking ligands, and evaluated their antiviral potency against the herpes simplex virus-2 (HSV-2). We found that hydrophobic scaffolds without charges exhibit stronger inhibition than hydrophilic ones with identical ligand number and density. The best candidate, B3C9S, also showed irreversible antiviral effect against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) both in vitro and in vivo, through a mechanism that involved virus envelope disruption and genome releasing. These results demonstrate the value of hydrophobic scaffolds in the development of multivalent virucidal antivirals.

316. Druggability Assessment of the Bunyaviral Cap-binding Domain

Janna Scherf, Ph.D. Seeking, *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
Dominik Vogel, Ph.D., *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*
Sheraz Gul, Ph.D., *Fraunhofer Institute for Translational Medicine and Pharmacology, Hamburg, Germany*
Philip Gribbon, Ph.D., *Fraunhofer Institute for Translational Medicine and Pharmacology, Hamburg, Germany*
Maria Rosenthal, Ph.D., *Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany*

The Bunyavirales order includes human pathogens like the severe fever with thrombocytopenia syndrome virus (SFTSV). Viral genome replication and transcription are catalysed by the large (L) protein, containing a cap-binding domain (CBD). Viral transcription requires a capped RNA primer obtained by cap-snatching via the CBD. Specific binding of a cap-analogue (m7GTP) to the SFTSV CBD was demonstrated and an analogous domain in influenza viruses was shown to be inhibited by small molecules. As cap-snatching is essential in the bunyavirus life cycle, the cap-binding function represents a valuable, potentially broad-spectrum drug target. By means of in vitro assays and medium-throughput screening of small-molecule libraries, we attempted to validate SFTSV CBD as an antiviral target. Fluorescence polarization (FP) and surface plasmon resonance (SPR) assays were developed to identify CBD specific ligands and microscale thermophoresis (MST) was used for validation. The overall assay strategies however failed, likely because m7GTP has only a moderate affinity to the CBD and does not constitute a reliable positive binding control. This is consistent with the m7GTP binding cavity being too shallow in a crystal structure of the complex to support classical in silico screening approaches. In conclusion, the weak interaction between the CBD and m7GTP in vitro made it difficult to develop robust screening assays and it was impossible to establish competition-based FP or SPR readouts. The SFTSV CBD displays limited tractability towards existing approaches based on small molecules and is therefore not, at present, a suitable target for classical in vitro or in silico drug discovery.

317. Efficay of Tecovirimat, Brincidofovir, and Cidofovir Against a Human Monkeypox Virus Isolate from the Currently Ongoing Epidemic in Europe

Graciela Andrei, Ph.D., *KU Leuven, Leuven, Belgium*
Tony Wawina, Ph.D., *KU Leuven, Belgium*
Robert Snoeck, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Piet Maes, Ph.D., *KU Leuven, Leuven, Belgium*

In May 2022, WHO declared an atypical outbreak of human monkeypox (hMPXV) caused by the West African clade. Since then, >25,000 confirmed cases have been reported in Europe and >80,000 globally. Although no specific treatments for patients suffering from hMPXV are licensed, drugs that have been developed for smallpox in preparedness for a potential bioterrorist attack can be used. These drugs include Tecovirimat (TPOXX or ST-246), an inhibitor of the viral envelope protein VP37 that blocks the release of extracellular enveloped viruses from infected cells, impeding virus spread within the infected host, and brincidofovir (Tembexa), a lipid conjugate analogue of cidofovir, that like its parental drug, inhibits viral DNA replication. We explored the efficacy and selectivity of these drugs against a hMPXV isolate from the currently ongoing epidemic in human embryonic lung (HEL) fibroblasts and Vero cells. Tecovirimat showed superior activity and selectivity compared to brincidofovir and cidofovir against a hMPXV virus isolate, being the EC50 values found for this clinical strain similar to previous values reported for reference MPXV strains (PMID: 35904001). The superior potency of Tecovirimat was confirmed by inhibition of extracellular virus production. Importantly, differences on the effects of tecovirimat on the yield of hMPXV-cell associated virus were identified between Vero and HEL cells. We have previously reported such differences for cowpox and camelpox when comparing HEL cells and primary human keratinocytes (PMID: 18240860). Our study supports the use of Tecovirimat as the drug of choice for treating complicated cases of human monkeypox.

318. Generation and Characterization of Recombinant MA-EBOV Expressing Reporter Proteins in vitro and in vivo for use in Therapeutic and Vaccine Efficacy Studies

Katherine Davies, Ph.D., *CDC, Atlanta, Georgia, United States*
Stephen R. Welch, Ph.D., *Viral Special Pathogens Branch, CDC, GA, United States*
Shilpi Jain, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Teresa E. Sorvillo, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, Georgia, United States*
JoAnn D. Coleman-McCray, *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Joel M. Montgomery, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Cesar G. Albarino, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Jessica R. Spengler, D.V.M., Ph.D., *CDC, Atlanta, Georgia, United States*

Small animal models of disease are useful tools for evaluation of therapeutics and countermeasures offering several advantages over large animal models, including cost, feasibility of larger group sizes, and safety. Wild-type Ebolavirus (EBOV) does not cause lethal infection in mice which necessitated the development of a mouse-adapted (MA-) EBOV

produced by repeated passage in mice. To advance the utility of MA-EBOV for disease and medical countermeasure studies, we developed recombinant variants that express reporter proteins to track virus dissemination within infected animals and serve as tools to assess anti-viral therapeutics. We generated recombinant MA-EBOV (rMA-EBOV) containing all the unique mutations identified in MA-EBOV, and rMA-EBOV expressing ZsGreen1 and/or Nano-luciferase. We performed in vitro characterization to evaluate replication kinetics and determine any detriment to virus growth resulting from inclusion of MA-EBOV associated mutations or from addition of reporter proteins and assessed the susceptibility of these rMA-EBOVs to a panel of anti-viral compounds. Finally, we characterized the rMA-EBOV variants in CD-1 mice. Mice were infected intraperitoneally with MA-EBOV, rMA-EBOV, or rMA-EBOV variants to verify that there is no alteration of the acute, lethal phenotype conferred by MA-EBOV. Clinical signs and weights were assessed daily, and viral tissue loads were assessed at terminal end point or at study completion (14 days post-infection) by quantitative PCR or titration. Furthermore, the utility of the reporter protein-labelled rMA-EBOV for visualization of virus localization was assessed at endpoint in vivo using advanced imaging techniques.

319. High-Throughput Fluorescent Assay for Inhibitor Screening of Proteases from RNA Viruses

Andrea Huskova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Bara Cihlova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Jiri Böserle, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Radim Nencka, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Praha, Czech Republic*
Jan Silhan, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

Since the beginning of this century, we were present in at least 60 severe epidemic outbreaks, mostly caused by RNA viruses such as Ebola, Zika, Yellow fever, Dengue viruses and coronaviruses, SARS, MERS and SARS-CoV-2. The virology and enzymology of these viruses have been studied extensively for the past several years. However, the spread of these viruses is causing pressure to quickly develop and test new effective antivirals, which is an essential step to eradicate or diminish the severity of viral diseases in mankind. Thus, several kinetic and structural studies of their enzymes are available [1,2,3].
We have designed a fast and affordable high-throughput assay based on fluorescent energy transfer (FRET) to test potential inhibitors [4]. The designed FRET molecule consists of eGFP–protease site–mCherry. If protease of interest is successfully inhibited, energy is transferred and light is detected. This robust and reproducible assay can be used for testing the inhibitors in 96- or 384-well plates.

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320. Identification and Evaluation of Novel Macrocyclic Compounds Against Hemorrhagic Fever Arenaviruses

Virginia Aida-Ficken, D.V.M., *The Centers for Disease Control & Prevention, Atlanta, GA, United States*
Jamie A. Kelly, Ph.D., *The Centers for Disease Control & Prevention, GA, United States*
Payel Chatterjee, M.S., *The Centers for Disease Control & Prevention, Atlanta, GA, United States*
Markus Kainulainen, Ph.D., *The Centers for Disease Control & Prevention, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Mike J. Flint, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Lassa virus (LASV), the causative agent of Lassa fever, is a persistent threat to public health, with an estimated 500,000 infections and 10,000 deaths reported annually across sub-Saharan west Africa. The epidemic potential and lack of licensed therapeutics for LASV necessitates the discovery and development of antiviral countermeasures, an effort that has been hampered by the requirement for Biosafety Level 4 (BSL-4) containment for work with infectious LASV.
The current study used high-throughput drug screening techniques to evaluate a library of 60,000 compounds for potential LASV inhibitors. Initial screening employed an Echo 550 acoustic liquid handler to add compounds to Huh7 cells. These cells were transferred into a BSL-4 laboratory and infected with a recombinant LASV engineered to express the fluorescent reporter protein ZsGreen. Three days post-infection, ZsGreen fluorescence was measured to identify compounds with anti-LASV activities. A viability assay was run on compound-treated, but uninfected cells concomitantly to identify cytotoxic compounds.
From this screen, several structurally related macrocyclic compounds were identified as hits and confirmed in dose-response studies. The most efficacious of these, #12895623, demonstrated activity against wild-type (non-reporter) LASV by high-content imaging analysis, and was capable of reducing titers of infectious LASV in cell culture by >4 logs. This compound was further evaluated to have activity against different clades of wild-type LASV. Given this compound's potential, the mechanism of action was explored using pseudotype particles bearing the LASV glycoprotein and LASV minigenome systems.

321. Implementation of a Preclinical Platform for the Evaluation of Antivirals During an Emergence Period: the Example of SARS-CoV-2

Jean-Sélim Driouich, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Franck Touret, Ph.D., *UVE : Aix-Marseille University -IRD 190-Inserm 1207-IHU Méditerranée Infection, France*
Antoine Nougairède, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Xavier de Lamballerie, M.D., Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*

In the face of the SARS-CoV-2 pandemic and its unprecedented repercussions, not only on human health but also on society, ecology and economy, there was an urgent need for effective antiviral therapeutics. Repurposed drugs were massively evaluated in clinical trials to palliate the lack of antiviral molecules against coronaviruses. Unfortunately, they have not been able to deliver on their promise, with the vast majority of these drugs failing to demonstrate a clinical efficacy. This could be due to the lack of a systematic approach for evaluating antivirals, based on a rigorous preclinical study, before clinical evaluation. During the pandemic, we have implemented a preclinical platform to test repurposed drugs to generate robust preclinical evidence to guide clinicians and health authorities for further clinical development. This translational drug development platform comprises in vitro, ex vivo, and in vivo models of SARS-CoV-2, along with a pharmacokinetic approach to evaluate exposure levels in plasma and target organs of treated animals. Here, we provide examples of repurposed drugs tested within our platform. Our data confirm the importance of in vitro, ex vivo, in vivo and pharmacokinetic evaluations of molecules, in multiple assays, to generate strong preclinical evidence to support clinical trials.

322. In vitro Evaluation of Double Combinations Against Coxsackievirus B3 and Poliovirus Type 1

Adelina Stoyanova, Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*
Simeon Galabov, M.D., *The Stephan Angeloff Institute of Microbiology, BAS, Bulgaria*
Stefan Philipov, Ph.D., *Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Sofia, Bulgaria*
Vadim Makarov, Ph.D., *Bach Institute of Biochemistry, RAN, Moscow, Russia*
Angel S Galabov, M.D., Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

The human enteroviruses (EV) cause a diverse array of clinical features, including aseptic meningitis, HFMD, neonatal sepsis-like disease, pancreatitis, encephalitis, myocarditis, paralysis, respiratory diseases, ect. The best known members are the polioviruses (PV) and coxsackieviruses. There are no EV-specific drugs available for clinical use. One of the reasons is fast development of drug-resistant mutants. Synergistic combinations of two agents can overcome toxicity and restrict the emergence of resistance to the partners in the combination. We studied the combined effects of inhibitors with different mode of action against Coxsackievirus B3 (Woodruff strain) (CV-B3) and Poliovirus-1 (LSc–2ab strain) (PV-

1). Theoretical additive interactions of expected effects for drug–drug interactions was calculated by using MacSynergy II. Interpretation of significance of the observed volumes of synergy or antagonism, depicted in the differential surface plots, was based upon the program guidelines. The combinations of pocapavir with oxoglaucine, 2-(alpha-hydroxybenzyl)-benzimidazole (HBB) and pleconaril agains CV-B3 demonstrated additive effect. The combination of pocapavir and oxoglaucine indicate synergistic antiviral activity on PV-1 replication in HEp-2 cells. An additive effect was exhibited when pocapavir was combined with 2-(alpha-hydroxybenzyl)-benzimidazole (HBB) and pleconaril against PV-1. Tested combinations had no cytotoxicity effects on uninfected HEp-2 cells.

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323. In vitro Screening for Inhibitors of the 2′-O-ribose Methyltransferase VP39 from Mpox Virus

Dominika Chalupska, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Karel Chalupsky, *Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Czech Republic*
Tomáš Otava, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Jan Silhan, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Radim Nencka, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Praha, Czech Republic*

In eukaryotic cells, mRNAs are protected from premature degradation by the nucleotide structure called a cap on the 5′ end. Viruses often also incorporate a cap structure at the 5′ end of their RNAs to protect themselves from the host innate immune system and to enhance protein synthesis. Some viruses exploit the host capping system, others use their own enzymes (1). Mpox virus is an emergent human pathogen. It is a member of the genus Orthopoxvirus within the Poxviridae family of double-stranded DNA (dsDNA) viruses. Mpox virus encodes its own enzymes for cap synthesis. These include VP39, mpox 2′-O-methyltransferase, which adds a methyl group at the 2′-O location of the proximal ribose of the initial nucleotide, creating the mature cap. This step prevents the development of innate immune responses, so VP39 could be used as a possible drug target within antiviral treatments. We developed a method coupled with ECHO-MS system for measuring methyltransferase activity and used it to test activity of 2′-O-RNA methyltransferase from the OC43 coronavirus (2). Later we optimized the method for high throughput screening and used it for identifying inhibitors of mpox VP39 with IC50 approximately 10 times lower than that of sinefungin, a potent pan-methyltransferase inhibitor.

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324. Treatment via Consecutive Alternating Administration of Antivirals Against Coxsackievirus B3 Infection in Mice

Adelina Stoyanova, Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*
Nikoleta Hristova, B.S., *The Stephan Angeloff Institute of Microbiology, BAS, Bulgaria*
Simeon Galabov, M.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*
Vadim Makarov, Ph.D., *Bach Institute of Biochemistry, RAN, Moscow, Russia*
Stefan Philipov, Ph.D., *Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Sofia, Bulgaria*
Gerhard Pürstinger, Ph.D., *Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria*
Georgi Dobrikov, Ph.D., *Institute of Organic Chemistry with Centre of Phytochemistry, BAS, Sofia, Bulgaria*
Angel S Galabov, M.D., Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

Human enteroviruses, distributed worldwide, are causative agents of a broad spectrum of diseases with high morbidity, including a series of severe illnesses that affect the CNS, heart, skeletal muscles, and so on. There is no specific treatment available for these infections, and the patients' treatment is mainly supportive. Our team has developed an experimental treatment strategy based on consecutive alternating application (CAA) of enteroviral inhibitors. This work represents the antiviral activity of triple combinations of anti-enteroviral compounds applied via CAA course against Coxsackievirus B3 (Woodruff strain) (CV-B3) infection in newborn mice. Antiviral combination effects were examined by relying on triple CAA combinations consist of pleconaril (Pl), pocapavir (Po) and vapendavir (V) (Pl/Po/V) or pleconaril(Pl), MDL-860 (M) and oxoglaucine (O) (Pl/M/O) against CV-B3 infection in ICR newborn mice infected s.c. with 20 MLD50. Cumulative mortality (percentage), mean survival time (MST) (days) and weight (in grams) of suckling mice were recorded. The results of these analyses indicate improved efficacy of Pl/M/O combination administered according to the CAA treatment schedule in CV-B3 infected mice - decreased mortality rate and lengthening of the mean survival time (MST). Pl/Po/V applied consecutively were ineffective. In comparison with placebo groups the monotherapeutic course with pleconaril demonstrated some independent antiviral effect. It was found that pocapavir, vapendavir, MDL-860 and oxoglaucine monotherapies were without a protective effect.

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325. In vitro Studies of the Barrier to Resistance of Sofosbuvir Against Tick-borne Encephalitis and Yellow Fever Viruses

Alekxander Binderup, M.S., *Copenhagen Hepatitis C Program (CO-HEP), University of Copenhagen and Hvidovre Hospital, Copenhagen, Denmark*
Lotte S. Mikkelsen, *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Ulrik Fahnøe, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Jens Bukh, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Santseharay Ramirez, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre, Hvidovre, Denmark and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Tick-borne encephalitis virus (TBEV) and yellow fever virus (YFV) are re-emerging flaviviruses that cause high morbidity in humans with an increasing number of yearly infections despite available vaccines, with no approved antiviral treatments. Using concentration-response assays, we found that nucleotide analog sofosbuvir, used for hepatitis C virus treatment, can inhibit both TBEV and YFV at non-cytotoxic and clinically relevant concentrations in human hepatoma Huh7.5 cells with 50% drug effective concentrations (EC50) of 4.2 and 2.6 μM, respectively. TBEV and YFV drug escape variants selected in sofosbuvir long-term treatment experiments exhibited reduced sofosbuvir susceptibility (2.2- and 7.1-fold increased EC50 respectively). In addition, sofosbuvir longer-term treatment efficiently suppressing the original TBEV could not control infection with the drug escape variant. Using next-generation sequencing of the complete open reading frame of the escape viruses, we identified several substitutions at high frequency in the drug target, the RNA dependent RNA polymerase domain of NS5. For TBEV, these were Y453H, I569M, S603T, F701C or F701L. The frequency of S603T decreased after drug free passage and S603T caused a significant loss of fitness in reverse-genetics experiments. For YFV, NS5 substitutions M478K and A482G were maintained at high frequency after drug free passage suggesting no effect on viral fitness. Our results demonstrate that sofosbuvir exhibits a high barrier to resistance against TBEV in vitro. The clinical benefit of repurposing sofosbuvir for the treatment of TBEV should be addressed in clinical trials as currently being investigated for YFV.

326. Inhibitors of Coronavirus and Monkeypox Virus Methyltransferases: Similarities and Differences

Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic*

RNA methyltransferases (MTases) synthesize the 5′ RNA cap which is important for the stability of the RNA and for efficient translation (1). During the last two years, many inhibitors of coronaviral MTases were prepared by us and others (2-5). We have solved a series of crystal structures of these inhibitors in complex with the coronaviral nsp14 and nsp16/nsp10 MTases (6, 7). Recently we also solved the crystal structure of monkeypox virus MTase VP39 in complex with the pan-MTase inhibitor sinefungin and with several inhibitors. These structures revealed significant differences between coronaviral and poxviral MTases but also revealed that these enzymes can be targeted by the same or very similar compounds.

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327. Leveraging the Power of Artificial Intelligence to Discover Drugs for Pandemic Preparedness

Fraser Cunningham, Ph.D., *Exscientia, Dundee, United Kingdom*
Alberto Bresciani, Ph.D., *Exscientia, United Kingdom*
Marco Derudas, Ph.D., *Exscientia, United Kingdom*
Simon Richards, M.S., *Exscientia, Oxford, United Kingdom*
Jake McGreig, Ph.D., *Exscientia, United Kingdom*
Ian Goodfellow, Ph.D., *Exscientia, Oxford, United Kingdom*
Thorsten Novak, *Exscientia, Oxford, United Kingdom*

The current COVID-19 pandemic has highlighted an urgent need for broad spectrum antiviral therapeutics to limit the impact of future pandemics. This presents the challenge of developing such molecules at a time when the precise nature of the pathogen is unknown. Furthermore, such molecules should be safe for the larger population, easy to administer and have a high therapeutic adherence, blocking transmission and hence avoiding species adaptation and variant generation.

We aim to overcome these challenges with an approach enabling the development of molecules with broad spectrum activity, which are active against pathogens with a high potential to cause pandemics, and with a high barrier to resistance. To achieve this, we have built dedicated AI systems that efficiently learn from a diverse range of data and consistently reapply enhanced knowledge through iterative design processes. Our AI platform learns more effectively and rapidly than human-led efforts alone, and as such, candidate molecules satisfying complex therapeutic requirements suitable for pandemic preparedness can be created with improved efficiency. Our goal is for our AI platform to revolutionise the development of antivirals for pandemic preparedness.

In this poster, we will illustrate how our AI platform works and how we are creating a portfolio of projects to discover and develop drug candidates against viruses with pandemic potential.

328. Mechanisms of Action of Repurposed Ebola Virus Antivirals

Jamie Kelly, *Centers for Disease Control and Prevention, Atlanta, GA, United States*
Virginia Aida, *Centers for Disease Control and Prevention, GA, United States*
Payel Chatterjee, M.S., *The Centers for Disease Control & Prevention, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Mike J. Flint, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Repurposed drug screens are a useful method to identify potential antiviral therapeutics. This approach has identified many approved drugs that can inhibit highly lethal diseases like Ebola virus (EBOV) infection in cell culture, yet few prove to be successful in vivo. Phospholipidosis (PLD) is a side effect of many cationic amphiphilic drugs resulting in excess lipid accumulation in the cell. Studies repurposing FDA-approved drugs as SARS-CoV-2 antivirals found many PLD-inducing compounds assessed in vitro did not mediate protection against infection in vivo, suggesting PLD could be a confounding factor in repurposing screens. Though the exact mechanism is not known, induction of PLD may be a non-specific adverse drug effect resulting in 'false positive' hits in cell-based antiviral screens. To address this issue and optimize the drug discovery process, we aimed to identify compounds with anti-EBOV activity that did not induce PLD and then determine the mechanism of their antiviral activity. We compiled a list of nearly 400 hit compounds from nine anti-EBOV screens. Over half of the selected compounds were eliminated because they were predicted to induce PLD,

based on their structural and chemical properties as well as previous reports in the literature. From the remaining hits, we selected a panel of anti-EBOV compounds for mechanistic studies using lentiviral pseudotype particles bearing the EBOV glycoprotein and an EBOV minigenome system to investigate viral entry and replication, respectively. Antiviral efficacy was confirmed in live EBOV infection assays at BSL-4. We report the identification of several candidate anti-EBOV drugs with mechanisms, distinct from PLD.

329. Modeling SARS-COV-2-infected Central Nervous System Using Human Primary Neuronal/Glial Cells to Identify Antiviral Drugs

Noemie Berry, Ph.D., *INRAE, Maisons-Alfort, France*
Nazim Ahnou, M.S., *INSERM, France*
Rozenn Brillet, M.S., *INSERM, Créteil, France*
François Piumi, M.S., *INRAE, Maisons-Alfort, France*
Valentine Chaillot, M.S., *INRAE, Maisons-Alfort, France*
Dennis Salomon Lopez-Molina, Ph.D., *INSERM, Créteil, France*
Odile Blanchet, M.D., *CHU Angers, Angers, France*
Nathalie Aulner, Ph.D., *Institut Pasteur, Paris, France*
Anne Danckaert, Ph.D., *Institut Pasteur, Paris, France*
Hakim Ahmed-Belkacem, Ph.D., *INSERM, Créteil, France*
Muriel Coulpier, Ph.D., *INRAE, Maisons-Alfort, France*

SARS-CoV-2 induces a large range of neurological symptoms even without respiratory manifestations. However, there is still no consensus concerning viral entry and viral tropism in the brain as well as viral-induced inflammation. Here, we used a well-characterized culture of human neuronal/glial cells (hNGCs) differentiated from fetal neural progenitors to provide new arguments about SARS-CoV-2 infection in human brain. We showed that astrocytes were highly permissive to the virus, confirming Andrews et al., (2022) who used human cortical organotypic slices and brain organoids. In hNGCs, viral infection led to a strong alteration of neuronal morphology and to astrocytes death. SARS-CoV-2 infected-hNGCs were then used to screen twenty molecules (viral polymerase inhibitors, statins, antimalaria or antiparasitic drugs, etc…) with either already known or unknown antiviral activity against this virus. Image analyses, quantification of viral RNA and viral particles were used to determine the anti-viral efficiency. We revealed that seven molecules with known anti-SARS-CoV-2 activity in other cell types were inefficient in hNGCs whereas the others were confirmed, which demonstrated that the efficiency of these molecules was cell-type dependent. Among previously unknown antiviral molecules against SARS-CoV-2, we found that small-molecule cyclophilin inhibitors have an antiviral activity in infected-hNGCs. Our data thus demonstrate that human astrocytes are permissive to the virus and that infection strongly affects human neuronal/glial cells. They also outline the importance of using cellular model that are brain specific to question the role of antiviral molecules in this organ.

330. Modulation of the Aryl Hydrocarbon Receptor Signaling Pathway Impacts on Junín Virus Replication

Miguel Ángel Peláez, Ph.D. Seeking, *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
Maria Florencia Torti, Ph.D. Seeking, *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
Aaron Ezequiel Álvarez De Lauro, Ph.D. Seeking, *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
Agostina Belén Marquez, Ph.D. Seeking, *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
Federico Giovannoni, Ph.D., *Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States*

Elsa Beatriz Damonte, Ph.D., *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*
Cybele Carina Garcia, *Laboratory of Antiviral Strategies, Biochemistry Department, School of Sciences, University of Buenos Aires, IQUIBICEN, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina*

Junín virus (JUNV), a member of the family Arenaviridae, is the etiological agent of Argentine hemorrhagic fever, an endemic disease in the rural region of Argentina lacking a specific chemotherapy. Aryl hydrocarbon receptor (AHR) is expressed in several mammalian tissues and has been indicated as a sensor of ligands from variable sources and modulator of the cell immune response. Interestingly, recent studies suggested that activation or depression of AHR signaling pathway may play a role in the outcome of diverse human viral infections. In the present report, the effect of the pharmacological modulation of AHR on JUNV in vitro infection was analyzed. An initial microarray screening showed that AHR gene was overexpressed in JUNV-infected hepatic cells. Concomitantly, the infection of Vero and Huh-7 cells with the JUNV strains IV4454 and Candid#1 was significantly inhibited in a dose-dependent manner by treatment with CH223191, specific antagonist of AHR, as detected by infectivity assays, real time RT-PCR and immunofluorescence detection of viral proteins. Furthermore, the pro-viral role of AHR in JUNV infection appears to be independent of the IFN-I pathway. Our findings support the promising perspectives of the pharmacological modulation of AHR as a potential target for the control of AHF.

331. Nebulization of Fusion Inhibitory Lipopeptide, A Way to Protect Nonhuman Primates Against Respiratory Nipah Virus Infection

Olivier Reynard, Ph.D., *CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS, UMR5308, Univ Lyon, Université Claude Bernard Lyon 1, École Normale Supérieure de Lyon, Lyon, Rhone, France*
Iampietro Mathieu, Ph.D., *CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS, UMR5308, Univ Lyon, Université Claude Bernard Lyon 1, École Normale Supérieure de Lyon, Rhone, France*
Dumont Claire, *CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS, UMR5308, Univ Lyon, Université Claude Bernard Lyon 1, École Normale Supérieure de Lyon, Lyon, Rhone, France*
Le Guellec Sandrine, *DTF-Aerodrug, R&D Aerosolltherapy Department of DTF Medical (Saint Etienne, France), Faculté de Médecine*, Université de Tours, *Tours, Indre et Loire, France*
Moroso Marie, Ph.D., *INSERM-Laboratoire P4 Jean Mérieux, Lyon, Rhone, France*
Stephanie Durand, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, Rhone, France*
Brisebard Elise, D.V.M., *UMR703, PAnTher APEX, INRAE/Oniris, Nantes, Loire Atlantique, France*
Cyrille Mathieu, Ph.D., *International Center for Infectiology Research (CIRI), Lyon, Rhone, France*
ChristopherA Alabi, Ph.D., *Robert Frederick Smith School of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, United States*
Vecellio Laurent, *PST-A, Université de Tours, Tours, Indre et Loire, France*
Matteo Porotto, Ph.D., *Department of Pediatrics, Center for Host-Pathogen Interaction, Columbia University Medical Center, New York, NY, United States*
Anne Moscona, M.D., *Department of Pediatrics, Center for Host-Pathogen Interaction, Department of Microbiology & Immunology, Department of Physiology & Cellular Biophysics, Columbia University Medical Center, New York, New York, United States*
Branka Horvat, M.D., Ph.D., *CIRI, Centre International de Recherche en Infectiologie, INSERM U1111, CNRS, UMR5308, Univ Lyon, Université Claude Bernard Lyon 1, École Normale Supérieure de Lyon, Lyon, Rhone, France*

Nipah virus (NiV) is a lethal zoonotic paramyxovirus that can be transmitted from person to person via the respiratory route. NiV causes encephalitis and severe respiratory disease and there is no licensed therapeutic currently available against this emerging infection. A lipopeptide-based fusion inhibitor strategy has been developed and previously evaluated for its efficacy against NiV (Malaysia strain) in vitro and in vivo, notably in hamsters and nonhuman primate model using intratracheal administration of peptides. To advance a clinically applicable method for respiratory administration of lipopeptides, we developed a mesh nebulizer and face mask and evaluated them in an artificial monkey breathing model to optimize delivery. The peptide aerosolization approach was than evaluated in an African green monkey model that reflects human NiV infection. Three consecutive aerosolized doses of the previously characterized lipopeptide, given 4 mg/kg every 24 hours, resulted in uniform lipopeptide distribution in the respiratory

tract. Peptide nebulization was not associated with evident allergic reaction, toxicity, or adverse hematological and biochemical effects. . When treated monkeys were intratracheally challenged with a lethal dose of NiV (Bangladesh strain), lethality was significantly delayed. This aerosol administration method supports the feasibility of the antiviral lipopeptide strategy, which we propose as the basis for an emergency response that can be implemented immediately upon identification of a new virus that infects via a Class 1 fusion mechanism. Aerosol delivery of fusion inhibitory peptides may be used to protect against NiV and other airborne viruses.

332. Non-nucleotide RNA-dependent RNA-polymerase Inhibitor as Potential Antiviral Drug

Katerina Krejcova, M.S., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic*
Eva Konkolova, Ph.D., *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic*

Coronaviruses (family Coronaviridae) and flaviviruses (family Flaviviridae) positive-sense RNA viruses that cause numerous important human and animal diseases, such as acute diarrhea, vomiting, encephalitis, acute flaccid paralysis, hemorrhagic fevers or congenital abnormalities and fetal death. Unfortunately, both families have proved that they hold pandemic potential, as demonstrated recently by the ZIKV, MERS-CoV and SARS-CoV outbreaks. Therefore, effective treatment strategies are urgently needed to treat patients infected with flaviviruses. The RNA-dependent RNA polymerase (RdRp) is a key enzyme in the replication of RNA viruses and therefore is the one of the most important targets for treatment of viral infection [1, 2, 3].
In this study, we tested the ability of helquat-like ligand PR-673 against coronaviral and flavivirus RdRps. We used in vitro polymerase assays to show that these compounds interfere with RNA syntheses performed by the RdRps. This research was funded by the project the National Institute Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.¨

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333. Peptoid Amphiphiles as Membrane Active Antivirals

Patrick Tate, B.S., *New York University, New York, New York, United States*
Vincent Mastrodomenico, M.S., *Loyola University Medical Center, Illinois, United States*
Bryan Mounce, Ph.D., *Loyola University Chicago Medical Center, Maywood, Illinois, United States*
Kent Kirshenbaum, Ph.D., *New York University, New York, New York, United States*

The development of durable new antiviral therapies is challenging, as viruses can quickly evolve to establish resistance and attenuate therapeutic efficacy. New compounds that selectively target conserved viral features are attractive therapeutic candidates, particularly for combatting newly emergent viral threats. The innate immune system features a sustained capability to combat pathogens through production of antimicrobial peptides (AMPs); however, these AMPs have shortcomings that preclude clinical use. The essential functional features of AMPs have been recapitulated by peptidomimetic oligomers, yielding effective antibacterial and antifungal agents. Here, we show that a family of AMP mimetics, called peptoids, exhibit direct antiviral activity against an array of enveloped viruses, including the key human pathogens Zika, Rift Valley fever, and chikungunya viruses. These data suggest that the activities of peptoids include engagement and disruption of viral membrane constituents. To investigate how these peptoids target lipid membranes we used liposome leakage assays to measure membrane disruption. We found that liposomes containing phosphatidylserine (PS) were markedly sensitive to peptoid treatment; in contrast, liposomes formed exclusively with phosphatidylcholine (PC) showed no sensitivity. In addition, chikungunya virus containing elevated envelope PS was more susceptible to peptoid-mediated inactivation. These results indicate that peptoids mimicking the physicochemical characteristics of AMPs act through a membrane-specific mechanism, most likely through preferential interactions with PS.

334. Plitidepsin is a Host-Directed Antiviral that Transiently Inhibits Protein Translation of Distant Viruses while Shaping a Protective Proteostatic Cellular Response

Nuria Izquierdo-Useros, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Daniel Perez-Zsolt, *IrsiCaixa AIDS Research Institute, Spain*
Elisa Molina Molina, Ph.D. Seeking, *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Joan Josep Bech-Serra, Ph.D., *Josep Carreras Leukaemia Research Institute, Barcelona, Spain*
Roger Badia, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Eva Riveira-Muñoz, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Edurne Garcia-Vidal, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Martin Sachse, Ph.D., *Instituto de Salud Carlos III, Madrid, Spain*
Marçal Gallemí, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Jordana Muñoz-Basagoiti, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Sandra Franco, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Sara Y. Fernández-Sánchez, Ph.D., *Centro Nacional de Biotecnología, CSIC, Madrid, Spain*
Dalia Raïch-Regué, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Cristina Lorca-Oró, Ph.D., *Unitat mixta d'investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA, Barcelona, Spain*
Raquel Tenorio, Ph.D., *Centro Nacional de Biotecnología, CSIC, Madrid, Spain*
Isabel Fernández de Castro, Ph.D., *Centro Nacional de Biotecnología, CSIC, Madrid, Spain*
Jorge Carrillo, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Julià Blanco, Ph.D., *IrsiCaixa AIDS Research Institute, Belona, Spain*
Alejandro Losada, Ph.D., *PharmaMar S.A, Madrid, Spain*
Pablo Aviles, *PharmaMar S.A, Madrid, Spain*
Carmen Cuevas, Ph.D., *PharmaMar S.A, Madrid, Spain*
Júlia Vergara-Alert, Ph.D., *Unitat mixta d'investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Barcelona, Spain*
Joaquim Segalésj, Ph.D., *Unitat mixta d'investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Barcelona, Spain*
Miguel Ángel Martínez, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Roger Paredes, M.D., Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Bonaventura Clotet, M.D., Ph.D., *IrsiCaixa AIDS Research Institute, Barcelo, Spain*
Cristina Risco, Ph.D., *Centro Nacional de Biotecnología, CSIC, Madrid, Spain*
Ester Ballana, Ph.D., *IrsiCaixa AIDS Research Institute, Barcelona, Spain*
Carolina de la Torre, Ph.D., *Josep Carreras Leukaemia Research Institute, Barcelona, Spain*

Different viruses employ similar pathways for replication that reveal key intracellular hot spots to target with host-directed therapies and achieve a broad-spectrum antiviral activity. Plitidepsin is a clinically approved antitumoral agent that blocks the elongation factor eEF1A required for protein translation. This drug impairs SARS-CoV-2 replication and shows a favorable safety profile in COVID-19 patients. Yet, the precise antiviral mechanism of action of plitidepsin remains unknown. Here we used deep quantitative proteomic analyses coupled to transmission electron microscopy and functional in vitro assays to dissect its antiviral activity against SARS-CoV-2. We also tested the antiviral effect of plitidepsin against other viruses, including Zika virus, replicon of Hepatitis C virus, Herpes simplex virus, and HIV-1. Plitidepsin inhibited the synthesis of all SARS-CoV-2 proteins, including R1AB involved in double membrane vesicle formation needed for viral replication. Yet, less than 14% of the cellular proteome was affected by plitidepsin, which up-regulated translation factors eIF4A2 and eIF2S3 associated to protein biosynthesis. At 50 nM, plitidepsin induced a compensatory proteostasis that rescued protein translation. Treatment also inhibited other RNA-dependent and non-integrated DNA viruses, such as Zika virus, Hepatitis C virus replicon, and non-lytic forms of Herpes simplex virus, but failed to block DNA integrated proviruses like HIV-1. Unraveling the mechanism of action of host-directed therapies like plitidepsin is imperative to develop broad-spectrum treatments and have them ready to deploy when future pandemic viruses break through.

335. Potent and Structurally Distinct Antiviral Hits Against Monkeypox Virus

Zhengqiang (ZQ) Wang, Ph.D., *Center for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, United States*
Lara Dsouza, Ph.D. Seeking, *Department of Veterinary Pathobiology, School of Veterinary Medicine & Biomedical Sciences, Texas A&M University, Texas, United States*
Jiashu Xie, Ph.D., *Center for Drug Design, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota, United States*
Anil Pant, Ph.D., *Department of Veterinary Pathobiology, School of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas, United States*
Subbian Satheshkumar Panayampalli, Ph.D., *Poxvirus and Rabies Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States*
Zhilong Yang, Ph.D., *Department of Veterinary Pathobiology, School of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, Texas, United States*

The ongoing monkeypox outbreak with a total of more than 80,000 cases reported in over 100 non-endemic countries, highlights the pandemic potential of poxviruses, and signifies the gravity of the threats posed by monkeypox virus (MPXV). Although typically manifesting in milder symptoms than smallpox, MPXV infections can still cause significant morbidities that need to be therapeutically mitigated. Also due to its zoonotic nature with broad hosts and unidentified natural reservoirs, future spillovers and outbreaks are expected even if the current outbreak is contained. The two drugs currently approved by FDA for stockpiling against smallpox, tecovirimat and brincidofovir (BCV), display suboptimal clinical efficacies, and are expected to drive the selection of drug-resistant mutants. Therefore, there is a need for structurally novel and mechanistically distinct antivirals for effectively treating MPXV infections. Towards this end, our multi-institutional team has identified and characterized two strong antiviral hits. Our hits inhibited VACV in the low to sub mM range in the phenotypic reporter assay and strongly reduced viral titer in the plaque assay. Significant inhibition against MPXV was also observed. In the in vitro ADME assays, our hits showed favorable profiles in plasma stability, liver microsomal stability and PAMPA permeability. Collectively, our data validate these hits as candidates for developing antivirals against MPXV.

336. Pre-Clinical Evaluation and Mode of Action of Molnupiravir against SARS-CoV-2

Paul-Rémi Petit, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Franck Touret, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, France*
Jean-Sélim Driouich, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Géraldine Piorkowski, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Caroline Laprie, *Laboratoire Vet-Histo, Marseille, France*
Laurent Fraisse, *Drugs for Neglected Diseases initiative, Geneva, Switzerland*
Peter Sjö, *Drugs for Neglected Diseases initiative, Geneva, Switzerland*
Charle E. Mowbray, *Drugs for Neglected Diseases initiative, Geneva, Switzerland*
Fanny Escudié, *Drugs for Neglected Diseases initiative, Geneva, Switzerland*
Ivan Scandale, *Drugs for Neglected Diseases initiative, Geneva, Switzerland*
Eric Chatelain, *Drugs for Neglected Diseases initiative, Geneva, Switzerland*
Xavier de Lamballerie, M.D., Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Caroline Solas, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Antoine Nougaigède, *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*

The SARS-CoV-2 pandemic has highlighted the need for broad-spectrum antiviral drugs to respond promptly to viral emergence. We conducted a preclinical study of molnupiravir (MOV) against SARS-CoV-2 to fully characterize its antiviral properties and mode of action. For this purpose, the antiviral activity of different concentrations of MOV was evaluated ex vivo on human airway epithelium (HAE) 2-4 days post infection (dpi) and in vivo in the hamster model at 3 escalating doses (150, 300 and 400 mg/kg/day) and according to 3 different regimes (preventive, preemptive and

curative). We assessed viral loads (VL) and infectious titer (IT) at the apical pole of HAE and in hamster lungs. To explore the mode of action of the MOV, the entire genomes of the collected viruses were sequenced in order to identify minority variants that represent at least 1% of the virus population. In HAE, concentrations of 1 and 3µM showed significant reductions of VL from 3 dpi and of IT from 2dpi. In vivo, all pre-emptive doses tested significantly decreased lung IT at 3dpi compared to untreated animals. When giving 300mg/kg/D of MOV as curative and preventive treatment, only the latter significantly reduced IT in lungs. Subpopulation analysis showed a rise of minority variants in treated animals, a significant increase of the transistion/transversion ratio and in the frequency of G to A mutations. The antiviral activity of MOV on SARS-COV 2 is more pronounced on the reduction of IT than on VL. This decrease in infectivity appears to be linked to a mutagenic effect in relation to its mechanism of action as a cytidine analogue, leading to G to A mutations.

337. PROTAC Approach for Antiviral Drug Discovery

Xicheng Sun, Ph.D., *Crestone Inc., Boulder, CO, United States*
Cliff Mason, Ph.D., *Crestone Inc., CO, United States*
Hang Liu, Ph.D., *Crestone Inc., Boulder, CO, United States*
Teresa Hoang, B.S., *Crestone Inc., Boulder, CO, United States*
Thale Jarvis, Ph.D., *Crestone Inc., Boulder, CO, United States*
Mary Ann DeGroote, M.D., *Crestone Inc., Boulder, CO, United States*
Josh Day, Ph.D., *Crestone Inc., Boulder, CO, United States*
Wendy Ribble, B.S., *Crestone Inc., Boulder, CO, United States*
Urs Ochsner, Ph.D., *Crestone Inc., Boulder, CO, United States*

Proteolysis targeting chimeric (PROTAC) is a new strategy used to degrade target proteins through the ubiquitin-proteasome system (UPS). Studies utilizing PROTAC demonstrated the feasibility of this approach on degradation of target proteins involved in tumor growth. The first oral PROTACs, ARV-110 and ARV-471, have shown encouraging results in clinical trials for prostate and breast cancer treatment, generating enthusiasm for PROTAC research. The application of PROTAC has been successful in cancer-related therapy with more than a dozen drug candidates entering clinical investigation, however, this approach has yet to be fully applied in antivirals. Recent studies suggest PROTACs may be a new tool in fighting pathogenic viruses by inducing the degradation of viral protein targets. The advantage of this approach in development of antivirals include depleting viral target proteins could result in the eradication of viruses and the prevention of resistance, recurrence or rebound. In theory, PROTAC molecules could be recycled to continue the degradation process, leading to enhanced killing kinetics of the viruses. In addition, unlike host proteins where some level of activity is needed for proper physiological function, there is no impact on host systems with complete degradation of viral proteins. We targeted the main protease of SARS-COV-2 by linking approved drugs to E3 ligase binders. We explored the length and geometry of the linker. Several PROTAC compounds demonstrated potent activities against coronaviruses with good selectivity in cells. Continued efforts to improve potency and drug-like properties through chemical modification of the linker are underway.

339. RNA Helicase eIF4A Inhibitors as Host-targeting Pan-antivirals

Arnold Grünweller, Ph.D., *Philipps University Marburg, Institute of Pharmaceutical Chemistry, Marburg, Germany*
Christin Müller, Ph.D., *Germany*
Francesca Magari, Ph.D., *Germany*
John Ziebuhr, M.D., Ph.D., *Germany*
Susanne Schiffmann, Ph.D., *Germany*

Inhibition of protein synthesis has become a promising antiviral strategy since all viruses depend on the translation machinery of the host cell. The RNA helicase eIF4A, a key component of the eukaryotic translation initiation complex eIF4F, is essential for replication of a wide range of RNA viruses. The presence of stable RNA structures in the 5′-untranslated mRNA regions of many of these viruses requires the unwinding activity of eIF4A.

Rocaglates are a class of potent and specific eIF4A inhibitors with broad-spectrum antiviral activity and a promising preclinical safety profile. They efficiently inhibit viral protein synthesis by clamping the 5′-UTRs of viral and selected cellular mRNAs onto the surface of eIF4A. This RNA-clamping prevents unwinding of RNA secondary structures thereby strongly impairing viral replication. Besides the natural rocaglate silvestrol, synthetic rocaglates like zotatifin or CR-1-31-B have been developed. So far, the most promising rocaglate is zotatifin since it has reached phase 1-2 in clinical trials.

Here, we will report on (i) the potent and non-toxic broad-spectrum antiviral activities of rocaglates against human Coronaviruses in an ex vivo lung epithelial cell model, (ii) mechanistic aspects of RNA-clamping in the context of viral 5′-UTRs, (iii) data from ADMET studies, and (iv) we will present an immunomodulatory profile of rocaglates. Taken together, our data show that eIF4A is an excellent target for the development of broad-spectrum antiviral drugs. This host-targeting strategy might have significant potential to help treat newly emerging RNA viruses more effectively in future outbreak situations.

340. Small Molecule Entry Inhibitors of Ebola and Marburg Filoviruses

Malaika Argade, Ph.D., *UICentre, College of Pharmacy, University of Illinois, Chicago, Chicago, IL, United States*
Laura Cooper, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States*
Ryan Bott, B.S., *University of Illinois Chicago, Chicago, Illinois, United States*
Mario Alvarez, *University of Illinois Chicago, Chicago, Illinois, United States*
Justin J. Patten, *Boston University, Boston, MA, United States*
Norton P. Peet, Ph.D., *Chicago BioSolutions Inc., Chicago, Illinois, United States*
Robert A. Davey, *National Emerging Infectious Diseases Laboratories, Boston University, Boston, MA, United States*
Terry W. Moore, Ph.D., *University of Illinois Chicago, Chicago, Illinois, United States*
Lijun Rong, Ph.D., *University of Illinois Chicago, Chicago BioSolutions Inc., Chicago, Illinois, United States*
Irina N. Gaisina, Ph.D., *University of Illinois Chicago, Chicago BioSolutions Inc., Chicago, Illinois, United States*

Filoviruses such as Ebola (EBOV) and Marburg (MARV), classified as Category A Bioterrorism Agents by the CDC, have gained worldwide attention due to their highly contagious nature and rapid progression of deadly hemorrhagic fever diseases. Since 1976, these viruses have been the cause of multiple disease outbreaks with the most widespread and recent ones being the 2014-2016 Ebola epidemic in West Africa and the 2022 Sudan-EBOV outbreak in Uganda, respectively. Despite having alarmingly high fatality rates (up to 90%), drug discovery efforts directed towards effective treatments for EBOV and MARV diseases continue to be understudied. Although the U.S. Food and Drug Administration has approved vaccines, Inmazeb and Ebanga for treating EBOV disease in 2020, there is still a critical need to develop novel small molecule antivirals to treat EBOV outbreaks and other infectious filoviruses. By virtue of our drug discovery efforts and high throughput screening strategy, we have identified 4-(aminomethyl)benzamide-based small molecules as potent entry inhibitors of EBOV and MARV, as tested in our pseudoviral assay. Through extensive SAR studies, we have successfully designed compounds that are potent (EC50<1 μM) and selective entry inhibitors of both EBOV and MARV, while possessing improved metabolic stability and low cytotoxicity (SI>100). As part of our ongoing medicinal chemistry efforts, we are focusing on optimizing our lead antivirals that, while retaining favorable potency and in vitro metabolic stability, will also exhibit desirable druglike properties.

341. Structural Characterisation of the Monkeypox Poxin In an Unliganded Form and Bound to a Novel Cyclic Dinucleotide MD1203

Vojtech Duchoslav, Ph.D. Seeking, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic*

Poxins are nucleases that specifically cleave the cyclic GMP-AMP (cGAMP) molecule. cGAMP is produced by the cGAMP synthase upon detection of DNA in cytoplasm. It is an important second messenger as it activates STING (stimulator of the interferon genes) that is involved in the innate immune response to viral infections in humans. Poxins were identified in the family Poxviridae which includes variola virus (smallpox) and vaccinia virus (used in the smallpox vaccine). These nucleases play a role in the virus's ability to evade the host's immune response by degrading cGAMP thereby eliminating of reducing the activation of the STING-mediated antiviral innate immune response. This study presents a structural characterization of the monkeypox poxin in an unliganded form and bound to a novel cyclic dinucleotide MD1203 that cannot be cleaved by poxins and thus has the potential to become an antiviral drug. This research was funded by the project the National Institute Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103) - Funded by the European Union - Next Generation EU.

342. Synergistic inhibitory Effect of Remdesivir and Ribavirin Against SARS-CoV-2

Carlos García-Crespo, Ph.D. Seeking, *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Ana Isabel De Ávila, B.S., *Centro de Biología Molecular Severo Ochoa, Spain*
Isabel Gallego, B.S., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Maria Eugenia Soria, B.S. Seeking, *Department of Clinical Microbiology, IIS-Fundación Jiménez Díaz, Madrid, Spain*
Brenda Martínez-González, Ph.D. Seeking, *Centro Nacional de Biotecnología, Madrid, Spain*
Lucía Vázquez-Sirvent, B.S., *Department of Clinical Microbiology, IIS-Fundación Jiménez Díaz, Madrid, Spain*
Pilar Somovilla, Ph.D., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Antoni Durán-Pastor, B.S., *Centro Nacional de Biotecnología, Madrid, Spain*
Cristina Ferrer-Orta, Ph.D., *Instituto de Biología Molecular de Barcelona, Barcelona, Spain*
Nuria Verdaguer, Ph.D., *Instituto de Biología Molecular de Barcelona, Barcelona, Spain*
Esteban Domingo, Ph.D., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Celia Perales, Ph.D., *Centro Nacional de Biotecnología, Madrid, Spain*

Background. Antiviral treatments against SARS-CoV-2 are currently under development. We previously described that favipiravir and ribavirin exert a synergistic lethal mutagenesis on hepatitis C virus (HCV) during replication on human hepatoma Huh-7.5 cells, and synergy was required to extinguish high fitness HCV. Therefore, we are interested in identifying synergistic combinations of lethal mutagens which are effective against SARS-CoV-2.

Methods. Vero E6 cells cells were infected at a MOI of 0.001 PFU/cell with SARS-CoV-2 (isolate WA1/2020, obtained from BeiResources). Synergy has been documented using a broad concentration range of remdesivir and ribavirin. Drug concentrations and inhibitory activities were entered in the CompuSyn software to apply the Chou-Talalay method and the SynergyFinder software, to identify a possible synergistic activity.

Results. The results document a synergistic activity by the combination of remdesivir and ribavirin acting on SARS-CoV-2. The quantifications show average dose reduction indices above 1, and average combination indices below 1 using the CompuSyn software. Similar results were obtained by using SynergyFinder with a ZIP score above 10. Other SARS-CoV-2 variants are under study to expand these results.

Conclusions. The combination of remdesivir and ribavirin shows synergistic antiviral activity against SARS-CoV-2. This may help to reduce the effective concentration of compounds to achieve a beneficial clinical effect. Synergy among nucleoside analogues may be a potential strategy to confront emerging viral infections.

343. Synthesis of Novel Antiviral Nucleoside Phosphoramidates Targeting Viral Polymerases

Jacob Sawyer, Ph.D. Seeking, *University of Minnesota, Minneapolis, MN, United States*
Samantha Kennelly, Ph.D. Seeking, *University of Minnesota, Minneapolis, MN, United States*
Daniel Harki, Ph.D., *Department of Medicinal Chemistry and Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, United States*

SARS-CoV-2, the causative agent of the worldwide COVID-19 pandemic, has underscored the need for new and effective antiviral agents. Therapeutic targeting of viral polymerases is a clinically proven and effective method for controlling virus growth. Recently, 3′-deoxy-3′,4′-didehydro-cytidine-5′-triphosphate (ddhCTP) was identified as the endogenous therapeutic nucleotide produced by the antiviral protein viperin. ddhCTP elicits antiviral activity through chain termination due to the absence of a hydroxyl group at the 3′ carbon of the ribose sugar, which is required for nucleic acid extension. The nucleoside, ddhC, was developed into a cell-permeable prodrug (ProTide) that can produce ddhCTP in cells through enzymatic activation. The resulting prodrug elicited broad-spectrum activity against Zika and West Nile virus. This work is expanding upon these results through the synthesis and antiviral testing of the related endogenous nucleotide analogues. This presentation will cover our unpublished efforts to synthesize 3′-deoxy-3′,4′-didehydro-U/T/A/G/I nucleosides, as well as their corresponding phosphoramidate prodrugs. Screening of these compounds for antiviral activity against select arboviruses is currently underway. Additional synthesis efforts are focused on preparation of the corresponding 5′- triphosphates of these nucleosides for enzymology studies with viral polymerases. This work serves as the underpinning for the development of improved antiviral agents for the treatment of current and future viral outbreaks.

344. The Human DEAD-box RNA Helicase eIF4A as a promising Pan-antiviral Target - Mechanistic Aspects and Fragment-based Development of New eIF4A Inhibitors

Francesca Magari, Ph.D., *Philipps-Universität Marburg, Marburg (Lahn), Germany*
Henning Kutzner, *Philipps-Universität Marburg (Lahn), Germany*
Wiebke Obermann, *Philipps-Universität Marburg, Marburg (Lahn), Germany*
Leonie Konopka, *Philipps-Universität Marburg, Marburg (Lahn), Germany*
Henri Messner, *Philipps-Universität Marburg, Marburg (Lahn), Germany*
Alois Fürstner, *Max-Planck-Institut für Kohlenforschung, Mülheim an der Ruhr, Germany*
Martin Schlitzer, *Philipps-Universität Marburg, Marburg (Lahn), Germany*
Andreas Heine, *Philipps-Universität Marburg, Marburg (Lahn), Germany*
Arnold Grünweller, *Philipps-Universität Marburg, Marburg (Lahn), Germany*

The RNA helicase eIF4A, which is part of the translation initiation complex eIF4F, unwinds stable RNA structures in 5`-UTRs of selected mRNAs. As protein synthesis of a large number of RNA viruses requires eIF4A, this helicase is a promising target for the development of pan-antiviral compounds. It is already known that rocaglates like silvestrol and CR-31-B (-) inhibit RNA virus replication at low nanomolar concentration by a mechanism called RNA-clamping. However, the exact mode of action and which amino acids are relevant for the binding of rocaglates are not fully understood. We therefore investigated the mode of action of rocaglates by combining molecular docking with Thermal Shift Assay and mutational approaches. Based on our studies, an Arginine pocket seems to play a crucial role in eIF4A-RNA complex formation which is an essential step for proper inhibition by rocaglates and for the helicase activity of eIF4A. Interestingly, pateamines are a compound class that, although chemically different from rocaglates, can functionally mimic RNA-clamping. We therefore investigated pateamines for their RNA-clamping efficiency in several eIF4A variants to test their potential drug sensitivity and resistance in comparison to rocaglates.

Since pateamines and rocaglates have complex structures which are difficult to synthesize, our goal is the development of eIF4A inhibitors that can be obtained more easily. Based on screening of various fragment libraries and combining docking with experimental approaches, we have identified first hit candidates that are currently being optimized by fragment-growing approaches.

345. Towards Immunotherapy Against Alphaviral Encephalitis

AmandaL Phelps, *Defence Science Technology Laboratory (Dstl), Salisbury, United Kingdom*
Thomas R. Laws, *Dstl, Salisbury, United Kingdom*

Severe encephalitic alphavirus infections are in the most part, characterised by the pathology associated with inflammation of the brain. Despite being an immune-privileged organ, the blood brain barrier is known to alter, allowing conventional inflammation to occur. While the inflammation process is undoubtedly complex, there are a spectrum of licensed drugs to modulate it, both at a general and at the specific pathway level. Treating disease-associated pathology with immunotherapies was successfully demonstrated when used during the SARS-CoV-2 pandemic. Both dexamethasone and tocilizumab were used to alleviate the most severe cases of COVID-19. In this study, we used a mouse model of the biothreat agent Venezuelan Equine Encephalitis Virus to characterise the nature of the brain inflammation and relate this to the associated pathology. We found substantial upregulation of multiple specific targets for immunomodulatory drugs and general Th1- biased inflammation, which correlated well with levels of observed pathology. Specifically, this coincided with increased numbers of CD45+ leukocytes and raised titres of TNF-α, IFN-γ, IL1-α, IL-6 and IL-10 and multiple chemokines (CCL2, CCL5 and CXCL1). Infection in the brain and clinical presentation was most severe immediately prior to the lethal endpoint for these animals. In future studies, the use of already licensed drugs that target these inflammatory markers, will be assessed for their effect on the inflammatory response in this mouse model. The hope is that the use of immunotherapy strategies can be used to reduce the debilitating effects that this family of biothreat agents have on people.
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346. Unraveling the Mechanism of Action of the Broad-spectrum Antiviral Peptide Labyrinthopeptin A1

Merel Oeyen, Ph.D., *KU Leuven Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Leentje Persoons, *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Roderich Süssmuth, *Technische Universität Berlin, Germany*
Dominique Schols, Ph.D., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*

Viral infections form a tremendous risk to the global population. Recent epidemics highlight the importance of a broad-spectrum antiviral drug that is quickly available when a novel epidemic occurs.
We have previously demonstrated that the lantibiotic Labyrinthopeptin A1 (Laby A1) possesses antiviral activity against a broad range of enveloped viruses by binding to the viral phospholipid membrane, thereby inhibiting virus entry (Prochnow et al., 2020, e01471-19; Oeyen et al., 2021, 10.1016/j.virol.2021.07.003). Laby A1 inhibits various flaviviruses, all evaluated HIV and herpesvirus strains and isolates, as well as a broad range of respiratory viruses such as RSV and coronaviruses. However, in some cases this antiviral activity appears to be strain- and/or cell type-dependent, as we demonstrate using phenotypic and cellular impedance-based assays. We further unravel the reasons of this variable activity by exploring if the viral entry mechanism or the lipid content of the viral or cellular membranes affect Laby A1's activity.

Multiple attempts to obtain a Laby A1-resistant HIV-1 or dengue 2 virus strain failed, pointing to a high resistance barrier. Currently, we aim to obtain a Zika virus strain exerting resistance to Laby A1, and perform intermediate sequencing of the viral genome to gain further insight into the mechanism of action of this peptide. To conclude, we believe that with further optimization and (pre)clinical validation, this unique peptide has the potential to become a broad-spectrum agent against (novel) emerging viruses.

347. Unveiling Antiviral Mechanisms of Plitidepsin by In-Silico Transcriptomic Analysis

Jose A. Lopez-Martin, M.D., Ph.D., *PharmaMar - Virology, Colmenar Viejo, Madrid, Spain*
Fernanda Meira, M.D., *PharmaMar - Virology, Madrid, Spain*
Elena-Maria Saiz-Lou, M.D., *PharmaMar - Virology, Colmenar Viejo, Madrid, Spain*
Raquel Lloris, Ph.D., *PharmaMar - Virology, Colmenar Viejo, Madrid, Spain*
Diego Lopez-Mendoza, M.D., Ph.D., *PharmaMar - Virology, Colmenar Viejo, Madrid, Spain*
Jose Jimeno, M.D., Ph.D., *PharmaMar - Virology, Colmenar Viejo, Madrid, Spain*

Plitidepsin is an EF1A-targeting marine antiviral peptide in clinical development (COVID19). No published transcriptional effects on infected cells are available. We explored public repositories to identify additional antiviral mechanisms. Data were retrieved from GEO repository (GSE5681), from SKI-DLCL cells (human diffuse large cell lymphoma), following 24 h-exposure to either plitidepsin 3 nM (IC50) or DMSO (control), using cDNA microarrays (Affymetrix chip HG U133A-2), in triplicate assays. (Humeniuk R, et al doi:10.1038/sj.leu.2404911). We used R 4.0.3 and packages affy, limma, clusterProfiler, DOSE, and enrichplot. Benjamini-Hochberg adjustment was used for multiple testing [significance (SIG) < 0.05]. Genes were ranked by log2 fold change (logFC) for gene-set enrichment analysis. Relevant (REL) gene-sets were defined by a Normalized Enrichment absolute Score greater than 1.96. Plitidepsin induced SIG differential expression in 4101 from 8018 distinct annotated genes: 15 with REL upregulation (logFC > 1) and 48 with REL downregulation (logFC < -1), resulting in SIG and REL changes in 90 GO (gene ontology) biological processes, not clearly related to the cell neoplastic condition. We highlight gene enrichments in negative regulation of viral genome replication, viral process or life cycle; response to type I interferon; negative enrichment in neutrophil activation and degranulation; negative changes in membranes and endoplasmic reticulum. Plitidepsin at very low nM conc. (clinically feasible) is able to induce transcriptional changes in an eukaryotic cell line, which might be related to the underlying mechanisms of its antiviral and anti-inflammatory properties

348. Viral Capsids as Tools for Structural Biology

Petr Škvára, Ph.D. Seeking, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic*

The goal of structural biology is to elucidate the structure of bio-macromolecules in atomic detail. Until now, the method of choice was X-ray crystallography. However, its bottleneck is the preparation of diffraction quality crystals. However, recently cryo-electron microscopy (cryoEM) that relies on imaging individual molecules using electrons is being used more and more [1]. CryoEM can reach the atomic resolution by combining imagines of thousands of molecules, however, it is itself limited by the size of the sample analyzed. Too small (<100 kDa) bio-molecules are difficult to align to access the atomic resolution. We plan to prepare pentamers of viral major capsid protein (VP1) derived from the mouse polyomavirus that would be able to harbor any target small protein. To produce such a versatile system, we plan to employ the cameloid nanobody that would be targeted against the protein of interest. Prepared pentamers will be analyzed using cryoEM and protein crystallography to demonstrate their ability as a useful tool for structural analysis of small proteins in cryoEM. Up until now, we were able to design, optimize and crystallize a stable protein complex of VP1 harboring the CFP as a model protein. This research was funded by project the National Institute of Virology and Bacteriology (Programme EXCELES, Project No. LX22NPO5103)–Funded by the European Union–Next Generation EU.
1. R. Henderson, et al. (1990), Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy
2. Chen, et al. (1998), Interaction of polyomavirus internal protein VP2 with the major capsid protein VP1 and implications for participation of VP2 in viral entry

349. Virucidal Drugs

Francesca Olgiati, M.S., *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Chiara Medaglia, Ph.D., *Université de Genève, Switzerland*
Pablo Gainza Cirauqui, Ph.D., *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Bruno Correia, Ph.D., *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Caroline Tapparel, *Université de Genève, Genève, Switzerland*
Francesco Stellacci, *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*

The world has changed during the last three years, due to pandemic caused by the spread of SARS-CoV-2 in China. We are always fighting against viruses, some that are new and emerging, but also some others that exists since hundreds of years and are still causing medical and economic impacts. For these reasons, it is important to develop new antiviral drugs that are broad spectrum and can be adapted to many different viruses with minimal modifications. Recently, our group has developed virucidal macromolecules that target the receptor binding domain of many viruses and can inhibit the viral replication through a permanent disruption of the capsid. The interaction established with the virus is unspecific and could potentially cause side-effects. In this work we show that the same macromolecules can be modified to target specifically different parts of viruses but maintaining the same mechanism of action. The specific binding is achieved through protein-protein interactions, with peptide sequences. The results show that targeting the hemagglutinin of influenza virus leads to irreversible in-vitro inhibition (EC50 as low as 0.1 ug/mL) and no cytotoxicity. The generation of a potentially universal platform of antiviral drugs will be proved by confirming the same approach with other viruses.

350. Efficacy and Safety of Zapnometinib in Hospitalized Adult Patients with COVID-19: A Randomized, Double-blind, Placebo-controlled, Multi-center, Phase 2 Trial (RESPIRE)

Stephan Stenglein, M.D., *Atriva Therapeutics GmbH, Germany*
Gernot Rohde, M.D., Ph.D., *Universitätsklinikum Frankfurt, Frankfurt, Germany*
Hans Prozesky, *TREAD Research, Tygerberg Hospital, Cape Town, South Africa*
Ganesh Manudhane, *Seven Hills Hospital, Mumbai, India*
Oana Sandulescu, *National Institute for Infectious Diseases "Prof.Dr. Matei Bals", Carol Davila University of Medicine and Pharmacy, Bucharest, Romania*
Martin Bauer, M.D., *Atriva Therapeutics GmbH, Tübingen, Germany*
Winfried Koch, *BDS Koch, Schwetzingen, Germany*
Oliver Planz, *Eberhard Karls University, Tübingen, Germany*
Antoni Torres, *Hospital Clinic i Provincial de Barcelona, Barcelona, Spain*
Martin Witzenrath, *Charité Universitätsmedizin Berlin, Berlin, Germany*

Zapnometinib (ATR-002) is a highly specific MEK1/2 inhibitor that demonstrated immunomodulatory and antiviral properties (dual effect) against SARS-CoV-2 in preclinical studies and was safe and well tolerated in healthy subjects. RESPIRE (NCT04776044) was a randomized, double-blind, placebo-controlled, phase 2 trial. Hospitalized adults with COVID-19 were randomized 1:1 to oral zapnometinib (900mg on Day 1; 600mg daily on Days 2–6) or matching placebo. The primary endpoint was clinical severity status (CSS) on a 7-point ordinal scale at Day 15; time to hospital discharge (TTHD) was the key secondary endpoint. The trial was terminated early as the emergence of the Omicron variant impacted recruitment. Overall, 104 patients were randomized and 101 were included in the full analysis set (zapnometinib: n=50; placebo: n=51). Baseline CSS was well balanced: 40.0% of patients on zapnometinib and 41.2% on placebo had a CSS of 4 (hospitalized, requiring supplemental oxygen). On Day 15, patients on zapnometinib had higher odds of improved CSS vs placebo (OR 1.54 [95% CI 0.72–3.33]; p=0.262). TTHD showed a trend in favor of zapnometinib (rate ratio 1.31 [0.81–2.13]; p=0.274). In pre-defined subgroup analyses, zapnometinib improved CSS vs placebo in patients with severe disease at baseline (CSS 4) (OR 2.57 [0.76–8.88]; p=0.128) and reduced median TTHD by 1.5 days (RR 1.59 [0.73–3.57]; p=0.245). Zapnometinib had a favorable safety profile: the incidence of serious and non-serious adverse events was low and comparable between arms. The clinical benefit of zapnometinib shown in RESPIRE provides proof of concept for the absolutely novel concept of MEK inhibition in infectious disease.

352. Oral 4′-Fluorouridine Protects Against Lethal Lassa Virus Infection In Guinea Pigs and Can Rapidly Improve Clinical Signs When First Dose Administered During Peak Febrile Period of Disease

Jessica R. Spengler, D.V.M., Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Stephen R. Welch, Ph.D., *Viral Special Pathogens Branch, CDC, GA, United States*
Payel Chatterjee, M.S., *The Centers for Disease Control & Prevention, Atlanta, Georgia, United States*
Katherine A. Davies, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, Georgia, United States*
JoAnn D. Coleman-McCray, *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Sarah C. Genzer, D.V.M., *Comparative Medicine Branch, CDC, Atlanta, Georgia, United States*
Teresa E. Sorvillo, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, Georgia, United States*
Florine Scholte, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Jillian Condrey, D.V.M., Ph.D., *Comparative Medicine Branch, CDC, Atlanta, GA, United States*
Brian Gowen, Ph.D., *Utah State University, Logan, United States*
Manohar Saindane, *Emory Institute for Drug Development, United States*
George R. Painter, Ph.D., *Emory Institute for Drug Development, United States*
Joel M. Montgomery, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*
Alexander A. Kolykhalov, Ph.D., *Emory Institute for Drug Development, Emory University, Atlanta, GA, United States*
Michael G. Natchus, Ph.D., *Emory Institute for Drug Development, Emory University, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Mike J. Flint, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Lassa fever (LF) is an acute hemorrhagic illness caused by Lassa virus (LASV), an arenavirus endemic in West Africa. Currently, no antivirals are licensed for LF, and supportive care remains the mainstay of treatment. Here, we investigated the efficacy of 4′-fluorouridine (4′-FlU, EIDD-2749), a ribonucleoside analog, given orally beginning 7 days post-infection (dpi) when widespread virus replication is detectable in the strain 13/N guinea pig model of lethal LASV disease. Animals received 5 mg/kg QD (daily) or QOD (every other day) for a 7 to 20 dpi treatment window and were monitored for up to 35 days post-infection. In addition, a subset of animals was euthanized at 16 dpi for clinical and virological analyses. No clinical signs were observed in any animals receiving EIDD-2749, whereas all untreated controls met humane euthanasia endpoint criteria due to severe disease (at 20–25 dpi). At 16 dpi, a 2–4 log reduction of vRNA levels in most tissues and swabs was observed. No infectious virus was found in tissues or mucosal specimens of any treated animals. Subsequent studies evaluated reduced dosing and delayed treatment. Three lower doses delivered QOD beginning at 7 dpi similarly prevented, or improved, clinical signs. Furthermore, two experimental groups given 5 mg/kg QD or QOD beginning at the peak of the febrile period (at 12 dpi), resulted in signs of rapid clinical recovery after delivery of just a single dose. These data support EIDD-2749 as a highly effective therapeutic that can be given orally on a feasible dosing schedule and is efficacious even when the first dose is administered during the onset of clinical signs.

353. Single Dose Mucosal Delivery of a Nipah VRP-Based Vaccine Confers Rapid Protection Against Lethal Disease

Stephen R. Welch, Ph.D., *CDC, Atlanta, Georgia, United States*
Jessica R. Spengler, D.V.M., Ph.D., *CDC, Georgia, United States*
Sarah C. Genzer, D.V.M., *Comparative Medicine Branch, CDC, Atlanta, Georgia, United States*
JoAnn D. Coleman-McCray, *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Jessica Harmon, M.S., *CDC, Atlanta, Georgia, United States*
Teresa E. Sorvillo, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, Georgia, United States*
Florine Scholte, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Sergio Rodriguez, Ph.D., *CDC, Atlanta, Georgia, United States*
Jana Ritter, D.V.M., Ph.D., *CDC, Atlanta, Georgia, United States*
Georgia Ficarra, Ph.D., *CDC, Atlanta, Georgia, United States*
Katherine A. Davies, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, Georgia, United States*
Markus Kainulainen, Ph.D., *The Centers for Disease Control & Prevention, Atlanta, Georgia, United States*
Elif Karaaslan, Ph.D., *CDC, Atlanta, Georgia, United States*
Eric Bergeron, Ph.D., *CDC, Atlanta, Georgia, United States*
Cynthia Goldsmith, Ph.D., *CDC, Atlanta, Georgia, United States*
Michael Lo, Ph.D., *CDC, Atlanta, Georgia, United States*
Stuart Nichol, Ph.D., *CDC, Atlanta, Georgia, United States*
Joel M. Montgomery, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Nipah virus (NiV) is a highly pathogenic viral zoonoses with a 50–80% case fatality rate. Currently, there are no approved therapeutics or vaccines for NiV infection. Here, we generated a NiV virus replicon particle (VRP) in which the fusion protein (F) gene, essential for viral propagation, has been deleted. We demonstrate in vitro and in vivo that the VRP (termed NiVΔF) can replicate in the initial cells entered but cannot spread beyond due to lack of de novo synthesis of F, and that transient detection of NiVΔF in tissues is not associated with pathology. This VRP has broad utility for diagnostics, reagent generation, assay validation, drug discovery, and vaccination. We evaluated use of NiVΔF as a novel vaccine in highly sensitive small animal models demonstrating both safety and immunogenicity of the platform, which induced a broad antibody response against multiple viral antigens (NP, G, and F). In hamsters, single-dose intranasal (IN) vaccination 28 days prior to NiV challenge elicited 100% protection against all clinical signs in both IN and intraperitoneal (IP) models of infection. Protection was also conferred by short-course, single-dose vaccination up to 3 days prior to challenge. Efficacy was also shown in the IFNAR-/- mouse model of disease. Mice were vaccinated IP and challenged 7, 14, and 28 days post vaccination; none of the vaccinated mice demonstrated clinical signs post-challenge. These data support continued pre-clinical evaluation of the NiV VRP vaccine platform building on previous work by our group showing utility of the platform for a variety of high-consequence pathogens, and describe the development of a safe and critical tool for the NiV field.

354. Evaluation of Remdesivir and Monoclonal Antibody m102.4 Combination Against Nipah virus in Human Primary-like Small Airway Epithelial Cells in vitro

Michael Lo, Ph.D., *US Centers for Disease Control and Prevention, Atlanta, GA, United States*
Christopher Broder, Ph.D., *Uniformed Services University, MD, United States*
Danielle Porter, Ph.D., *Gilead Sciences, Inc., Foster City, CA, United States*
Joel M. Montgomery, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Nipah virus (NiV) is a zoonotic pathogen responsible for causing fatal encephalitis and acute respiratory disease in humans with high mortality (~ 40-75% CFR), and is designated a priority pathogen by the World Health Organization for research and development based on its epidemic potential and the relative lack of approved countermeasures for treatment in humans. Although several henipavirus glycoprotein-based vaccine platforms have shown promising efficacy in animal models including in nonhuman primates, only monoclonal antibody m102.4 and nucleotide analog remdesivir

(RDV) have shown therapeutic or post-exposure prophylactic efficacy in nonhuman primates, respectively. In this study, we evaluated in vitro combination treatment with these two therapeutics against Nipah virus infection in a primary-like small airway epithelial cell line using two assay platforms: 1) a reporter-based assay based on the NiV Malaysian genotype, and 2) a cytopathic effect-based assay based on the NiV Bangladesh genotype. Using the Multi-dimensional Synergy of Combinations (MuSyC) framework tool within the SynergyFinder online software package identified synergistic potency shifts for m102.4 and RDV across both assay platforms. Our results warrant further investigation of such combination treatments against NiV.

355. Development and Utilization of a Novel Minigenome and recombinant Vesicular Stomatitis Virus Expressing Seoul Hantavirus Glycoprotein-based Assays to Identify Promising Anti-hantavirus Therapeutics

Punya Shrivastava-Ranjan, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*
Shilpi Jain, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, GA, United States*
Payel Chatterjee, M.S., *The Centers for Disease Control & Prevention, Atlanta, GA, United States*
Joel M. Montgomery, Ph.D., *Center for Disease Control and Prevention, Atlanta, GA, United States*
Mike J. Flint, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Cesar G. Albarino, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Seoul virus (SEOV) is an emerging rodent borne hantavirus that can cause severe disease, known as hemorrhagic fever with renal syndrome (HFRS), and results in human case fatality rates of approximately 2%. There are no approved treatments for SEOV infections. To identify potential antiviral compounds for SEOV, we developed a cell-based high-throughput screening assay. Additionally, we generated the first reported minigenome assay for SEOV, to test if candidate antiviral compounds targeted viral transcription/replication, and a recombinant vesicular stomatitis virus expressing SEOV glycoproteins, to identify compounds that targeted SEOV glycoprotein-mediated entry. Altogether, we identified 9 compounds with robust anti-SEOV activity, including several with previously reported activity against other negative-strand RNA viruses. Our findings have important implications for the development of anti-SEOV therapeutics.

356. A Comparative Analysis of Host Response and Inhibition Amongst Diverse Ebolavirus Species

Laura K McMullan, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
M. Harley Jenks, B.S., *Virus Special Pathogens Branch, Centers for Disease Control, GA, United States*
Shilpi Jain, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Cesar G. Albarino, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Mike J. Flint, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*

Viruses in the genus Ebolavirus (EBOV) are genetically diverse and can cause a spectrum of disease, ranging from subclinical to fatal hemorrhagic fever. The selection driving the genetic diversity is thought to be due to adaptation to each EBOV host species, which are mostly unknown. The frequency of filovirus spillover into human populations in Africa and the recent discovery of novel EBOV species emphasize the need for therapeutics. Using authentic full-length viruses in the BSL-4 lab, we compared different EBOV species for their ability to infect, replicate, and induce a host response in primary target cells, human macrophages. We measured the viral transcriptional profile of different EBOV species and compared the host transcriptional response associated with EBOV replication. Host pathways enriched in response to EBOV replication included inflammation, proliferation, adhesion, NF-KB and interferon signaling. We screened a small molecule library targeting host pathways that were found enriched in the transcriptional analysis. Compound inhibitors specific for Src kinase, epidermal growth factor receptor, PI3K/Akt, and selective estrogen receptor modulators were compared between the different EBOV species for their effect on infection. Using constitutively-active and dominant-negative kinase mutants from these pathways, we compare EBOV species infection and gain insight in host restrictive and permissive pathways of filovirus infection and improve potential targets for intervention.

357V. Viral Replication and Host Immune Responses Early After Challenge in Mice Vaccinated with Crimean-Congo Hemorrhagic Fever Virus Replicon Particles

Teresa E. Sorvillo, Ph.D., *Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, United States*
Elif Karaaslan, Ph.D., *CDC, Georgia, United States*
Florine Scholte, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Stephen R. Welch, Ph.D., *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
JoAnn D. Coleman-McCray, *Viral Special Pathogens Branch, CDC, Atlanta, GA, United States*
Sarah C. Genzer, D.V.M., *Comparative Medicine Branch, CDC, Atlanta, Georgia, United States*
Jana Ritter, D.V.M., Ph.D., *CDC, Atlanta, Georgia, United States*
Christina F. Spiropoulou, Ph.D., *Virus Special Pathogens Branch, Centers for Disease Control, Atlanta, GA, United States*
Eric Bergeron, Ph.D., *CDC, Atlanta, Georgia, United States*
Jessica R. Spengler, D.V.M., Ph.D., *CDC, Atlanta, Georgia, United States*

Crimean-Congo hemorrhagic fever virus (CCHFV) is a virulent human pathogen and WHO priority pathogen due to a lack of countermeasures, including efficacious vaccines. A CCHF viral replicon particle (VRP) has shown protective efficacy against homologous and heterologous challenge in a lethal infar -/- mouse model when given as a single dose one-month prior to infection; it also protects in short-course as a single dose 3 days prior to challenge. The mechanisms conferring protection and how they vary based on time of vaccination are unknown. Our study examines how viral load and host immune responses post-challenge correlate with CCHF VRP-mediated protection. Groups of ifnar-/- mice were vaccinated 28, 14, 7, and 3 days prior to CCHFV infection; cohorts were euthanized at 3 and 6 days post-infection (dpi). We found that vaccination at all timepoints significantly reduced CCHFV viral load, mucosal shedding, and markers of clinical disease compared to controls. Investigations of immunity using a nonspecific VRP (LASV VRP) revealed that short-course (D -3) vaccine protection could not be attributed to nonspecific innate immune responses. We further determined that vaccination 28 days prior to challenge induced the most robust immunity against CCHF as virus replication was controlled most effectively in this cohort. Robust anti-NP IgG antibody titers were detected in longer-course vaccine groups, however our data suggests that factors other than titer alone may be important for virus clearance after infection, including antibody quality and function (antibody-dependent complement deposition, ADCD).

358V. Atypical Mutational Spectrum of SARS-CoV-2 Replicating In the Presence of Ribavirin

Pilar Somovilla Crespo, Ph.D., *Centro de Biología Molecular Severo Ochoa (CBM-CSIC), Madrid, Spain*
Carlos García-Crespo, *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Spain*
Brenda Martínez-González, Ph.D. Seeking, *Centro Nacional de Biotecnología, Madrid, Spain*
María Eugenia Soria, *IIS-Fundación Jiménez Díaz, UAM, Spain*
Ana Isabel De Ávila, B.S., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Isabel Gallego, B.S., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Pablo Mínguez, *Spain*
Antoni Durán-Pastor, B.S., *Centro Nacional de Biotecnología, Madrid, Spain*
Cristina Ferrer-Orta, Ph.D., *Instituto de Biología Molecular de Barcelona, Barcelona, Spain*
Llanos Salar-Vidal, *Spain*
Mario Esteban-Muñoz, *Spain*
Sonia Zúñiga, *Spain*
Isabel Sola, *Spain*
Luis Enjuanes, *Spain*
Jaime Esteban, *Spain*
Ricardo Fernández-Roblas, *Spain*
Ignacio Gadea, *Spain*
Jordi Gómez, *Instituto de Parasitología y Biomedicina "López-Neyra" (CSIC), Spain*
Nuria Verdaguer, Ph.D., *Instituto de Biología Molecular de Barcelona, Barcelona, Spain*
Esteban Domingo, Ph.D., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*

Background. Antiviral treatments against SARS-CoV-2 are currently under development. Lethal mutagenesis is an antiviral approach that consists of extinguishing a virus by an excess of mutations acquired during replication in the presence of a mutagenic agent, often a nucleotide analogue. Ribavirin (1-β-D-ribofuranosyl-1-H-1,2,4-triazole-3-carboxamide) (Rib), a purine analogue, acts as a lethal mutagen against several RNA viruses. Despite the widespread use of Rib as an antiviral agent, quantifications of its inhibition of SARS-CoV-2, and possible alterations of the mutational spectrum it evokes on the virus are lacking.

Methods. Vero E6 cells cells were infected with SARS-CoV-2 (isolate WA1/2020, obtained from BeiResources) at a MOI of 0.001 PFU/cell. The mutant spectrum was analyzed by ultra-deep sequencing with a mutant frequency cut-off of 0.1%.

Results. Ribavirin exerts an inhibitory and mutagenic activity on SARS-CoV-2 infecting Vero cells. Deep sequencing analysis of the mutant spectrum of SARS-CoV-2 replicating in the absence or presence of ribavirin indicated an increase of the number of mutations, but not of deletions, and modification of diversity indices, expected from a mutagenic activity. Notably, the major mutation types enhanced by replication in the presence of ribavirin were A → G and U → C transitions, a pattern which is opposite to the dominance of G → A and C → U transitions previously described for most RNA viruses.

Conclusions. Ribavirin acts as a lethal mutagen of SARS-CoV-2. The results encourage extending preclinical trials to consider the use of ribavirin in combination therapies for severe COVID-19 infections.

359V. Broad Spectrum Inhibitors Targeting the SAM-binding Site of Viral Methyltransferases

Hongmin Li, Ph.D., *University of Arizona, Tucson, Arizona, United States*
Subodh Samrat, *University of Arizona, Tucson, Arizona, United States*
Qamar Bashir, *United States*
Yiding Huang, *University of Arizona, Tucson, Arizona, United States*
George Zheng, *United States*
Qing-Yu Zhang, *University of Arizona, Tucson, Arizona, United States*
Zhong Li, *University of Arizona, Tucson, Arizona, United States*

Viral MTases are attractive drug targets because of their essential roles in viral replication and evading host immune system. Identification of viral MTase inhibitors was hampered by the lack of proper chemical probe to develop non-radioactive HTS assay for large scale screening. In this work, we developed a high throughput screening (HTS) assay to target the SAM-binding site of viral methyltransferases (MTases), using FL-NAH, a fluorescent analog of SAM, the methyl donor. The fluorescent polarization (FP)-based assay is universal and can be used by any SAM-dependent MTase, including viral MTases from representative flaviviruses and SARS-CoV-2. In this work, we perform a small scale HTS using the FP-based assay and identified two candidate inhibitors that inhibited the binding of FL-NAH to the SAM-binding site of MTases of representative flaviviruses and SARS-CoV-2. We further characterized the inhibitors for their potency in inhibition of the MTase enzyme activity of SARS-CoV-2 NSP14 MTase, SARS-CoV-2 NSP16/10 MTase and representative flavivirus MTase, in inhibiting viral replication and cytotoxicity to human cells. We also demonstrated that these compounds directly bound to the viral MTases. Overall, we developed a universal HTS assay to identify SAM-binding site inhibitors and successfully verified the assay.

360V. Frog Skin AMPs: Promising Antiviral Peptides

Annalisa Chianese, Ph.D., *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*
Carla Zannella, Ph.D., *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*
Alessandra Monti, Ph.D., *Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Naples, Italy*
Nunzia Doti, *Institute of Biostructures and Bioimaging (IBB), National Research Council (CNR), Naples, Italy*
Anna De Filippis, *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*
Gianluigi Franci, *Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy*
Massimiliano Galdiero, *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*

Viral infections represent a serious threat to the world population and are becoming more frequent. However, most current antivirals are directed to inhibit specific viruses since these therapeutic molecules are designed to act on a specific viral target to interfere with a particular step in the replication cycle. Therefore, the search and identification of broad-spectrum antiviral molecules are necessary to ensure new therapeutic options. Recently, several studies on antimicrobial peptides (AMPs) identified them as promising antiviral agents. The AMPs, also known as host defense peptides (HDPs), represent an emerging class of therapeutic agents in several fields; they are used as antibacterial, antiviral, antifungal, antiparasitic, antioxidant, and anticancer agents. One of the natural sources of AMPs is represented by amphibian skin secretions. In this study, the antiviral activity of peptides AR-23, RV-23, Hylin-a1, and HS-1, derived from the secretion of the Rana genus, has been evaluated against a wide panel of viruses comprising enveloped, naked, DNA, and RNA viruses. Preincubation of peptides with viruses has determined a significant antiviral activity, demonstrating that they could disrupt the viral envelope, as confirmed by TEM. Furthermore, screened peptides act on the extracellular phases of the viral lifecycle by blocking the viral attachment and entry phases. Our results show possible novel applications of amphibian skin peptides in the field of antivirals. Further studies will focus on their specific mechanism of action to clarify the viral target on which the peptides act.

361V. Pre-Clinical Efficacy of Nanobodies as Passive Immunotherapeutics in the Golden Hamster Model of SARS-COV-2 Infection

Yu Cong, M.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Saurabh Dixit, Ph.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Maryland, United States*
Erin Kollins, B.S., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Eliza W. Choi, M.S., *Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, United States*
Russell Byrum, B.S., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Lou M. Huzella, D.V.M., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Donna L. Perry, D.V.M., Ph.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Elena Postnikova, Ph.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Amit Ranjan, Ph.D., *Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, United States*
Lokendrasingh V. Chauhan, M.S., *Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, United States*
David Drawbaugh Ii, B.S., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Kyra Hadley, B.S., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Kelly Wetzel, M.S., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Marisa St. Claire, D.V.M., Ph.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
W. Ian Lipkin, M.D., *Center for Infection and Immunity, Mailman School of Public Health,;Vagelos College of Physicians and Surgeons, Columbia University, New York, United States*
Lisa E. Hensley, Ph.D., *Zoonotic and Emerging Disease Research Unit, National Bio and Agro-Defense Facility, U.S. Department of Agriculture, Manhattan, Kansas, United States*

Connie S. Schmaljohn, Ph.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Michael R. Holbrook, Ph.D., *Integrated Research Facility at Fort Detrick, Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Fort Detrick, Frederick, Maryland, United States*
Thomas Briese, Ph.D., *Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, United States*

Mutations in the SARS-CoV-2 spike protein have decreased or led to the complete loss of protective efficacy of several monoclonal antibody products designed to treat COVID-19. To prepare for the emergence of future SARS-CoV-2 variants and other zoonotic coronaviruses, cross-protective therapeutics and vaccines with broader efficacy are needed. Recently, nanobodies (variable domains of heavy-chain antibodies), derived from a subset of camelid immunoglobulins that neutralize the virus, have been developed as passive immunotherapeutics. Llama-derived nanobodies have shown broad sequence variation that promises wider epitope recognition and robust efficacy against recently emerged SARS-CoV-2 variants. Two nanobody constructs that demonstrated potency against variants of concern in vitro were selected for further evaluation as therapeutics against SARS-CoV-2 in the golden hamster model. A dose of 10 mg/kg of one or the other nanobody construct was administered intranasally (IN) at 24 h prior to, and 24 h following, IN exposure to the Alpha or Delta variant of SARS-CoV-2. Sham-treated virus-exposed hamsters showed clinical signs consistent with disease along with concomitant weight loss, viral replication, and infectious virus in lung tissue. Infection was marked by interstitial pneumonia with consolidation that progressed from Day 3 to Day 7 post-exposure. Therapeutic efficacy of both nanobodies was identified in treated hamsters by comparatively lower clinical scores, little or no body weight loss, and improved lung histopathology. These findings demonstrate pre-clinical efficacy of the nanobody constructs evaluated here against the Alpha and Delta variants of SARS-CoV-2.

362V. Screening of Peptides Designed On Schmallenberg Virus Glycoproteins Able to Inhibit the Viral Infection

Carla Zannella, Ph.D., *University of Campania Luigi Vanvitelli, Naples, Naples, Italy*
Annalisa Chianese, *Italy*
Biagio Santella, *Italy*
Giuseppe Greco, *Italy*
Serena Montagnaro, *Italy*
Giuseppe Iovane, *Italy*
Ugo Pagnini, *Italy*
Rinaldo Grazioso, *Italy*
Carla Isernia, *Italy*
Anna De Filippis, *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*
Gianluigi Franci, *Department of Medicine, Surgery and Dentistry "Scuola Medica Salernitana", University of Salerno, Baronissi, Italy*
Matteo Porotto, *Italy*
Massimiliano Galdiero, *Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy*

Arthropod-borne viral diseases are spreading all over the world. Schmallenberg virus (SBV) appeared in central Europe during 2011, where rapidly spread all over Europe. The viral particle is surrounded by a membrane consisting of two glycoproteins, Gn and Gc, essential to drive the viral entry. Therefore, both Gn and Gc may represent a target for antiviral development. We investigated the inhibitory activity of overlapping peptides modelled on the amino acid sequences of the two glycoproteins. Cytotoxicity was evaluated on hamster kidney cells (BHK-21) at different concentrations starting by 200 μM via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. In a preliminary antiviral screening, cells were treated with each peptide at 100 μM and simultaneously infected by SBV. Five out of the Gc peptides reached the 50% of inhibition cut-off. None of the Gn peptides had a consistent inhibitory effect and no peptide toxicity was observed by the MTT assay at the concentrations used in our experimental conditions. To better investigate the mechanism of action of the five active peptides, different time of addition and temperature shift assays were performed. Gc peptides were able to target the viral particles, by preventing the early

stages of the infection, namely virus attachment and entry into the host cell. Finally, the secondary structure of peptides was determined via circular dichroism (CD), indicating that the two most active peptides (Gc30 and Gc49) showed a different structure. Altogether, these results suggested the possible direct involvement of Gc described domains in the process of virus fusion to develop novel therapeutic compounds.

363V. SRI-44249, a Novel Anti-Influenza Lead Compound That Is Active Against Influenza A Viruses and Targets RNA-Dependent RNA-Polymerase Function

Fahim Ahmad, Ph.D., *Southern Research, Birmingham, AL, United States*
Omar Moukha-Chafiq, Ph.D., *Southern Research, AL, United States*
Anish Vadukoot, Ph.D., *Southern Research, Birmingham, AL, United States*
John P. Tillotson, Ph.D., *Southern Research, Birmingham, AL, United States*
Narayan D Chaurasiya, Ph.D., *Southern Research, Birmingham, AL, United States*
Sixue Zhang, Ph.D., *Southern Research, Birmingham, AL, United States*
Donghui Bao, Ph.D., *Southern Research, Birmingham, AL, United States*
Yohanka Martinez-Gzegozewska, M.S., *Southern Research, Birmingham, AL, United States*
Lynn Rasmussen, M.S., *Southern Research, Birmingham, AL, United States*
Paige Vinson, Ph.D., *Southern Research, Birmingham, AL, United States*
Corinne E. Augelli-Szafran, Ph.D., *Southern Research, Birmingham, AL, United States*
Babu L. Tekwani, Ph.D., *Southern Research, Birmingham, AL, United States*
Kathy A Keith, M.S., *University of Alabama, Birmingham, AL, United States*
Stephanie Moore, Ph.D., *University of Alabama at Birmingham, Birmingham, AL, United States*
Richard J Whitley, M.D., *University of Alabama at Birmingham, Birmingham, AL, United States*

SRI-44249 was identified as a novel small molecule anti-influenza hit through high-throughput screening against H1N1 and H3N2 influenza A viruses. SRI-44249 was active against multiple strains of H1N1 and H3N2 with an EC50 in the range of 0.4 to 5.2 uM. The activity of SRI-44249 against multiple strains of influenza A virus was confirmed by immunofluorescence, cytopathic effect (CPE), and virus titer reduction (VTR) assays. SRI-44249 showed higher activity against H3N2 strains compared to H1N1 strains. SRI-44249 was not observed to have cytotoxic effects against several mammalian cell lines namely, MDCK, A549, and Vero cells at 30 uM concentration. SRI-44249 had acceptable solubility but was very unstable in mouse and human liver microsomes. Intraperitoneal (i.p.) dosing of mice with SRI-44249 showed an acceptable PK with T1/2 of 3.85 hrs. SRI-44249 showed significant inhibition of influenza RdRp activity in PB2 cap-snatching assay and influenza RdRp minigenome transcription analysis. The time-course CPE analysis showed SRI-44249 inhibited the influenza virus during the early stage of the infectious cycle. The Indirect immunofluorescence analysis of MDCK cells infected with H3N2 indicated that SRI-44249 down-regulated the expression of viral nucleoprotein (NP). The Western blot analysis of H3N2-infected MDCK cells treated with SRI-44249 revealed the time-dependent reduction of viral proteins NP and PB2. PB2 expression was completely suppressed at 72 hours post-treatment in H3N2 infected-MDCK cells. These studies suggest RdRp PB2 subunit is a potential therapeutic target for the anti-influenza action of SRI-44249
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364V. Targeting West Nile Virus Replication by Xanthine Inhibitors

Corinne Augelli-Szafran, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Omar Moukha-Chafiq, Ph.D., *Southern Research, Alabama, United States*
Theresa Nguyen, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Shuklendu Karyakarte, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Sixue Zhang, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Paige Vinson, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Lynn Rasmussen, M.S., *Southern Research, Birmingham, Alabama, United States*
Alec Hirsch, Ph.D., *Oregon Health and Science University, Portland, Oregon, United States*
Ashish Pathaka, Ph.D., *Southern Research, Birmingham, Alabama, United States*

West Nile Virus (WNV) is one of the emerging flavivirus transmitted by the Culex and Aedes species of mosquitoes. The lack of available vaccines or antiviral drugs to treat or prevent WNV infection poses a public health challenge. We developed a high throughput screen (HTS) against a WNV-induced cytopathic effect (CPE) and screened a total

of 197K unique compounds from which 22 hits were identified and then reconfirmed in the antiviral and virus titer reduction (VTR) assay. SRI-37776 was selected as the lead compound which showed potent antiviral inhibition (EC90 = 0.93 μM, CC50 = 12 μM, and 3.2 logs in VTR at 10 μM in HEK293 cells). To further improve the antiviral activity against WNV and address the cytotoxicity and poor drug-like properties of this lead compound, a structure-activity relationship (SAR) campaign was initiated on this SRI-37776 chemical series towards the optimization of improving potency and its drug-like properties. These efforts led to the identification of a xanthine analog, SRI-38841, which has an EC90 = 0.87 μM, CC50 = >30 μM, VTR = 3.5 logs at 10 μM in HEK293 cells, and acceptable drug-like properties. The SAR study this novel chemical series will be presented.

400. A Novel Approach to Bridge the Gap from Virology Research to New Antivirals

Andreas Kühbacher, Ph.D., *AiCuris Anti-infective Cures AG, Wuppertal, Germany*
Holger Zimmermann, Ph.D., *AiCuris Anti-infective Cures AG, Wuppertal, Germany*

Developing a new antiviral drug is an increasingly complex endeavor. Already the path from identification of a novel target up to a preclinical candidate requires close cooperation between several experts including basic scientists, drug development specialists, clinicians, and business experts. To prevent new promising concepts arising from academic research from being abandoned before they can prove their potential, more and especially earlier interaction and cooperation between basic research and drug development companies is crucial. This may be fostered, for example, by collaborations via joint research projects, corporate venture funds or incubator programs. Here we present AiCuris' collaborative approach to early innovation and drug development. Using the example of the AiCubator program, a pioneering approach to collaboration in the field of anti-infectives, we show how interaction between pharmaceutical companies and academic research groups can be fostered to discover and develop new antivirals. Both the approach and examples of ongoing projects will be presented.

401. Cell Culture Studies of the Barrier to Resistance of Broad-spectrum Antiviral Remdesivir against Hepatitis C Virus

Kuan Wang, Ph.D. Seeking, *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Carlota Fernandez-Antunez, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Laura Collignon, *Denmark*
Line Abildgaard Ryberg, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre, 2650 Hvidovre, Denmark and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Lotte S. Mikkelsen, *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Ulrik Fahnøe, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Kenn Holmbeck, *Denmark*
Jens Bukh, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Santseharay Ramirez, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre, 2650 Hvidovre, Denmark and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Failure of hepatitis C virus (HCV) treatment due to direct acting antiviral (DAA) resistance can compromise the efficacy of highly active HCV therapy. Repurposing of broad-spectrum antivirals such as remdesivir (RDV) could be of interest for treating such DAA-resistant HCV infections. We investigated the efficacy and barrier to resistance of RDV against HCV genotype 2 (J6/JFH1) in Huh7.5 cells. In dose-response assays, RDV was very potent, with effective concentration 50% (EC50) of 43.5 nanomolar (12-fold lower than sofosbuvir). An RDV escape virus selected after long-term culture with increasing RDV concentrations exhibited a 4.7-fold increase in EC50 compared to the original virus. Cross-resistance was observed to another 1'-cyano nuc (GS-6620; 6.3-fold increase in EC50), but not to sofosbuvir (only 2.1-fold increase in EC50). The RDV escape showed faster growth kinetics compared to the original virus. Escape correlated with the emergence of substitutions throughout the genome including E143Q, T179A, and M289L in the NS5B polymerase. All substitutions increased RDV EC50, particularly in combination, with E143Q as a major contributor to resistance in long-term treatment assays. While the M289L mutant retained fitness, T179A and particularly E143Q caused a significant loss of fitness, which was only partially restored in the E143Q/T179A/M289L virus. Accordingly, the E143Q/T179A/M289L virus was genetically unstable in vivo, losing the E143Q mutation in human liver chimeric uPA/SCID mice. In conclusion, RDV showed remarkable efficacy and a high barrier to resistance in cell culture. Clinical trials will be needed to evaluate the therapeutic relevance of remdesivir.

402. Combination of HBV Capsid/core Assembly Modulators (CAMs) Leads to a Long-lasting Antiviral Effect in vitro

Julien Pronost, M.S., *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France*
Julie Lucifora, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France*
Caroline Pons, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France*
Emilie Charles, *France*
Anna Salvetti, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, F-69007, Lyon, France*
David Durantel, Ph.D., *HepVir - CIRI - INSERM, Lyon, France*

Current treatments of Hepatitis B virus (HBV) chronic infections, based mainly on nucleoside analogues (NAs), are not sufficient to cure HBV infections mainly because there are inefficient on the circular covalently closed DNA (cccDNA), which is responsible for long-term persistence. To get an HBV cure, cccDNA should be either targeted physically or functionally. Capsid/core assembly modulators are currently being developed to complement NAs and foster a fastest loss of cccDNA in patients. It is expected that CAMs could replace NAs if potent, and safe enough ones are discovered; potency being instrumental to achieve the three modes of actions that have been so far described for CAMs (Lahlali et al. AAC 2018). Here, we describe a fourth MoA of CAMs that can be obtained in particular with a CAM-Ab (which induces aberrant capsid structures) and concerns HBV RNA biogenesis when the concentration/potency is high. Using Run-On experiments, we indeed show that nascent RNA synthesis is impaired, as a result of cccDNA transcription inhibition, and lead to reduced HBV RNA accumulation as well as downstream viral entities (HBsAg, viremia…). Interestingly we also demonstrate that combination of CAM-Ab with CAM-N (which makes empty capsids with normal structures) further amplifies this inhibitory phenotype, allowing a prolonged antiviral effect after treatment cessation (no or weak rebound), which is not obtained in respective monotherapies. This long-lasting inhibitory phenotype is due to a strong and long-lasting repression of cccDNA activity, which is being epigenetically/epitranscriptomically characterized. This opens new perspectives for antiviral HBV treatment.

403. Discovery of Small Molecule Antivirals Targeting Hepatitis B Virus Epsilon Element

Melissanne de Wispelaere, Ph.D., *Evotec ID (Lyon) SAS, Lyon, France*
Charlotte Blanc, *Evotec ID (Lyon) SAS, France*
Gilles-Olivier Gratien, *Evotec (France) SAS, Toulouse, France*
Anne Tuukkanen, *Evotec SE, Hamburg, Germany*
Juliette Lavaux, *Evotec ID (Lyon) SAS, Lyon, France*
Viviane Liziard, *Evotec (France) SAS, Toulouse, France*
Pauline Puel, *Evotec (France) SAS, Toulouse, France*
Priscillia Rebus, *Evotec ID (Lyon) SAS, Lyon, France*
Manon Jouvet, *Evotec ID (Lyon) SAS, Lyon, France*
Mateusz Mieczkowski, *Evotec SE, Hamburg, Germany*
Claudia Beato, *Aptuit (Verona) Srl, Verona, Italy*
Ghislaine Marchand, *Evotec (France) SAS, Toulouse, France*
Stephanie Versluys, *Evotec (France) SAS, Toulouse, France*
Antoine Alam, *Evotec ID (Lyon) SAS, Lyon, France*
Steffen Kaspar Grimm, *Evotec SE, Hamburg, Germany*

Hepatitis B virus (HBV) epsilon element is a highly conserved RNA structure present in the pregenomic RNA (pgRNA) and the viral mRNAs. This 60-nucleotides long element mediates several important roles in the infectious cycle through interactions with viral and host factors. Its structural integrity is essential for genome replication and packaging. The epsilon element is specifically recognized by the viral polymerase to form a ribonucleoprotein complex that is then encapsidated by the viral core protein to form nucleocapsids. An additional function of the epsilon element is to act as a template for the polymerase-primed reverse transcription of the pgRNA. Because genome replication only happens within the cytoplasmic nucleocapsid, agents targeting the epsilon element have the potential to block encapsidation and subsequent reverse transcription of the pgRNA. In this work, we set to discover RNA binders of the epsilon element,

through SEC-LC/MS based screening of a lead-like compound library. We identified several chemical series that displayed specific binding to HBV epsilon and exhibited antiviral activity in HBV-infected primary human hepatocytes. Structure Activity Relationship studies allowed us to improve the physicochemical and metabolic properties of the lead series and to deliver 8 hit compounds with significantly improved antiviral activities. We are currently completing the characterization of these hits through structural studies and further elucidation of their mode-of-action.

404. Pharmacological Evaluation of N-hydroxypyridinediones to Support Optimization as HBV Ribonuclease H Inhibitors

Molly Woodson, Ph.D. Seeking, *St Louis University, Department of Molecular Microbiology and Immunology, St Louis, MO, United States*
Mohamed Mottaleb, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, Missouri, United States*
Grigoris Zoidis, Ph.D., *University of Athens, Department of Pharmaceutical Chemistry, Athens, Greece*
John Tavis, Ph.D., *Saint Louis University, Saint Louis, MO, United States*

The N-hydroxypyridinediones (HPDs) suppress HBV replication by inhibiting the HBV ribonuclease H (RNase H). The most potent HBV RNase H inhibitors are HPDs with low cytotoxicity (EC50 <100 nM, selectivity indexes >1100), but little pharmacological characterization has been done. Our goal was to understand factors that modulate HPD potency and to assess their potential to cause drug-drug interactions. The 16 compounds tested to date were highly soluble (> 200 μM) with high Papps (> 1*10-6 cm/sec) in PAMPA at pHs 5 and 6.5, but not at pH 7.4. The two most potent compounds were tested in MDCK-MDR1 permeability assays. They had low Papps but were not actively effluxed (B-A/A-B < 2). Five HPDs were tested in microsome and hepatocyte assays, and all had t_ > 30 min and > 2 hr, respectively. Plasma protein binding studies are ongoing. To assess drug-drug interaction potential, 10 structurally diverse compounds were screened at 10 μM against CYP3A4, CYP2C19, and CYP2C9 in competitive inhibition assays. No compounds significantly inhibited CYP3A4 activity (< 25%), but all 10 significantly inhibited CYP2C19 and CYP2C9. CYP3A4 and CYP2C19 induction was assessed for four HPDs so far by quantifying changes in mRNA expression, and none significantly increased CYP3A4 or CYP2C19 expression (fold change <2). CYP2C9 induction studies are ongoing. These results indicate the HPDs have desirable ADME parameters for preclinical to clinical testing, and they help inform structure-activity relationship studies as the HPDs continue to be optimized towards clinical candidate HBV RNase H inhibitors.

405. HBV RNaseH Inhibitors Can Decrease Capsid Accumulation and May Induce an Interferon Response in HBV Replicating Cells.

Molly Woodson, Ph.D. Seeking, *Saint Louis University, Saint Lous, MO, United States*
Daniel Bradley, Ph.D. Seeking, *Saint Louis University, Saint Louis, MO, United States*
Qilan Li, Ph.D., *Saint Louis University, Saint Louis, MO, United States*
John Tavis, Ph.D., *Saint Louis University, Saint Louis, MO, United States*

Hepatitis B virus (HBV) is a hepatotropic partially double-stranded DNA virus that replicates by reverse transcription. The HBV ribonuclease H (RNaseH) degrades the pgRNA after it is used as a template for reverse transcription. Inhibiting the RNaseH causes accumulation of RNA:DNA heteroduplexes (RDH), rendering the (-) polarity DNA strand inert as a template for (+) polarity strand synthesis. RDHs are stiffer than dsDNA and would exert more force on the capsid, with longer RDHs exerting correspondingly more force. We hypothesize that this additional force would reduce capsid stability. HepDES19 cells replicating HBV were treated with RNaseH inhibitors from three chemotypes. Core levels were measured by western blot and capsid levels were monitored with native capsid blots from clarified lysates. RNaseH inhibitors from two of the three chemotypes reduced capsid accumulation in a compound-dependent manner. Premature capsid disruption could release viral genomic material into the cytoplasm, which could induce a type I interferon (IFN) response if the aberrant reverse transcription products made in the presence of RNaseH inhibitors are detected as PAMPs. Therefore, we treated HepG2-NTCP and HepDES19 cells replication HBV with RNaseH inhibitors and measured IFN-β transcript levels. The inhibitors increased IFN-β mRNA levels relative to β-actin mRNA in HBV replicating cells, characteristic of an IFN response. Studies are ongoing to expand on these observations. These experiments show that HBV RNaseH inhibitors reduce capsid accumulation and may induce a type I IFN response, potentially amplifying the antiviral effects of RNaseH inhibitors.

406. Hepatoma Cell Line Allowing Efficient Replication of Hepatitis B/C/D/E Viruses Can Be a Relevant Model for Drug Screening

Roxanne Fouille, Ph.D. Seeking, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*
Eloi R.Verrier, *INSERM, Université de Strasbourg, Institut de Recherche sur les Maladies Virales et Hépatiques, UMR_S1110, Strasbourg, France*
Caroline Pons, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*
Olivier Diaz, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*
Maud Michelet, *INSERM, U1052, Cancer Research Center of Lyon (CRCL), University of Lyon (UCBL1), CNRS UMR_5286, Centre Léon Bérard, Lyon, France, Lyon*
Thomas F.Baumert, *INSERM, Université de Strasbourg, Institut de Recherche sur les Maladies Virales et Hépatiques, UMR_S1110, Strasbourg, France, Strasbourg, France*
Vincent Lotteau, *Centre International de Recherche en infectiologie, Lyon, France*
Anna Salvetti, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*
David Durantel, Ph.D., *HepVir - CIRI - INSERM, Lyon, France*
Julie Lucifora, *CIRI, Centre International de Recherche en Infectiologie, Univ Lyon, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France*

The liver can be infected by several viruses such as hepatitis B (HBV), hepatitis D (HDV), hepatitis C (HCV), hepatitis E (HEV) viruses, leading to end-stage liver diseases. Cases of multiple infections have been reported especially in low-income countries. Currently, only HCV infection can be cured with antivirals. Although, the 4 viruses replicate into the same cell type (the hepatocyte), few data are available about their replicative interplay in case of multi-infections. We therefore engineered an HuH7.5-NTCP cell line, that can be partially re-differentiated into hepatocyte with particular cell culture conditions, and showed it can replicate the 4 viruses for at least 3 weeks. To determine if this cell line can be used to screen for novel broad antivirals, we tested several known antivirals in mono-infections conditions. We observed a strong antiviral effect of IFN-a on HCV, HDV and HEV, but not on HBV in this model. We confirmed the antiviral effect of RG7834 (an inhibitor of PAPD5/7, involved in the A/G-mixed tailing of RNAs) on HBV and showed that it additionally decreased HCV and HEV RNA levels, while increasing that of HDV. Interestingly, whereas we confirmed the antiviral effect of an FXR-agonist (GW4064) on HBV and HDV (that we initially identified in primary human hepatocytes), we found here that GW4064 also strongly inhibited HEV replication, while increasing that of HCV. Altogether, these data illustrate that a molecule inhibiting one virus may actually boost another one. To conclude, we set-up a new in vitro model allowing multi-infections with hepatitis viruses that can be used for drug screening.

407. High Activity of Remdesivir and Other Broad-spectrum Antivirals for the Treatment of Multidrug Resistant Hepatitis C Virus in vitro

Santseharay Ramirez, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Carlota Fernandez-Antunez, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Ulrik Fahnøe, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Lotte S. Mikkelsen, *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Judith M. Gottwein, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Andrea Galli, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Jens Bukh, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*

Emergence of multidrug resistance (MDR) can challenge the efficacy of hepatitis C virus (HCV) antiviral therapy, threatening global virus elimination programs. In cell culture experiments, we demonstrate that an HCV genotype 3a virus with resistance to protease- and NS5A-inhibitors, and to the nuc sofosbuvir (MDR-HCV) does not respond to treatment with the highly potent pangenotypic combination regimen glecaprevir/pibrentasvir/sofosbuvir. MDR-HCV and original viruses exhibit similar fitness during long-term infection albeit with slight delayed propagation for MDR-HCV due to decreased replication. HCV-MDR exhibits cross-resistance to 2'C-modified nucs, similar susceptibility to remdesivir and galidesivir, and enhanced susceptibility to the mutagenic nucs ribavirin, favipiravir, and molnupiravir, and to interferon (IFN) alpha-2b. Enhanced ribavirin activity is mediated primarily by sofosbuvir resistance associated

substitution S282T. Compared to the original, MDR-HCV infection leads to lower activation of myxovirus resistance gene 1, an IFN-induced protein, due to inefficient phosphorylation of protein kinase R. Remdesivir, currently approved for the treatment of COVID-19 but initially developed as an anti-HCV drug, inhibits original and MDR-HCV similarly, and it is highly active in combination with protease and NS5A inhibitors or with favipiravir. Remdesivir exhibits a remarkable barrier against resistance as escape viruses with decreased drug susceptibility cannot be obtained in selection experiments. In conclusion, we provide proof-of-concept for efficient inhibition of an MDR HCV variant by currently available broad-spectrum antivirals that could be rapidly repurposed.

408. Influence of Assembly Modulators on the Structure and Assembly Kinetics of Hepatitis B Virus Capsid

Kalouna Kra, Ph.D. Seeking, *Institut de Biologie Intégrative de la Cellule, CNRS, Université Paris-Saclay, CEA, GIF-SUR-YVETTE, Essonne, France*
Laetitia Gargowitsch, *Laboratoire de Physique des Solides (LPS), CNRS, Université Paris-Saclay, Essonne, France*
Jéril Degrouard, *Laboratoire de Physique des Solides (LPS), CNRS, Université Paris-Saclay, Orsay, Essonne, France*
Guillaume Tresset, *Laboratoire de Physique des Solides (LPS), CNRS, Université Paris-Saclay, Orsay, Essonne, France*
Stéphane Bressaneli, Ph.D., *Institut de Biologie Intégrative de la Cellule, CNRS, Université Paris-Saclay, CEA, Gif-Sur-Yvette, France*

Hepatitis B virus (HBV) is an enveloped virus with an icosahedral nucleocapsid. The constituent of the capsid, the Core protein, is a dimeric protein, which can self-assemble in vivo but also in vitro into capsids containing mainly 120 dimers. The CAMs (for capsid assembly modulators) that can disrupt HBV capsid assembly are antiviral molecules currently in clinical trials. They can either accelerate the assembly of seemingly normal, but empty, icosahedral capsids (CAM-E such as JNJ-632) or cause the formation of aberrant (larger and malformed) assemblies (CAM-A such as BAY 41-4107). Some CAM-A can also disrupt preformed capsids. However, the emergence of second- and third- generation CAMs with new mechanisms of action is redefining this classification. Much of what is known about the mechanisms of action of CAMs comes from biochemical and structural work with recombinant, C-terminally truncated Core (Cp149). Here, we will present our characterization of the structures formed by Cp149 in the presence of several CAM-A and CAM-E, including well-known and newer compounds. We combine cryo-electron microscopy (cryo-EM) with time-resolved small-angle X-ray scattering (TR-SAXS) to characterize the structures and kinetics of the formation of Cp149 assemblies at nanometer-to-atomic resolution. We establish that both the CAM-A and some CAM-E actually modify the structure and stoichiometry of assembled capsids, albeit in more subtle ways for CAM-E compounds. At equal concentrations, not all CAM-E induce the same structural alterations. Some lead to mixtures of deformed and perfect capsids allowing atomic resolution reconstructions.

409. Sofosbuvir as a Mild Mutagen for Hepatitis C Virus

Brenda Martínez-González, Ph.D. Seeking, *Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain*
Isabel Gallego, B.S., *Centro de Biología Molecular Severo Ochoa, Spain*
Josep Gregori, *Internal Medicine Hospital Universitari Vall d'Hebron, Spain*
María Eugenia Soria, *IIS-Fundación Jiménez Díaz, UAM, Spain*
Pilar Somovilla Crespo, *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Spain*
Ana Isabel De Ávila, B.S., *Centro de Biología Molecular "Severo Ochoa", Madrid, Spain*
Carlos García-Crespo, *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Spain*
Antoni Durán-Pastor, *Centro Nacional de Biotecnología (CNB-CSIC), Spain*
Carlos Briones, *Centro de Astrobiología (CAB, CSIC-INTA), Spain*
Jordi Gómez, *Instituto de Parasitología y Biomedicina "López-Neyra" (CSIC), Spain*
Josep Quer, *Internal Medicine Hospital Universitari Vall d'Hebron, Spain*
Esteban Domingo, Ph.D., *Centro de Biología Molecular Severo Ochoa, Madrid, Spain*
Celia Perales, Ph.D., *Centro Nacional de Biotecnología, Madrid, Spain*

Hepatitis C virus (HCV) causes acute and chronic infections, and about 70% of those that develop chronicity are at an increased the risk of liver cirrhosis. Sofosbuvir (SOF) is a key component of several current combination therapies for HCV infection. Here we describe that during HCV infection of human hepatoma cells in culture, HCV displays a mild mutagen character, in addition to its well established inhibitory activity. Deep sequencing analysis of HCV populations passaged in the presence of 800 nM SOF revealed an increase of the mutation frequency, reflected in a statistically significant excess of C to U transitions in the mutant spectrum of HCV populations, relative to populations passaged in

absence of SOF. This mutagenic effect went unnoticed when Sanger sequencing of molecular clones was used for the analyses. SOF exhibited reduced efficacy against high-fitness HCV populations, and, in that case, no excess of any type of mutation induced by SOF was observed. The results suggest the intriguing possibility that SOF might behave as a mild mutagen for HCV, depending on the fitness of the viral population.

410. Structure-Guided Engineering of Active Hepatitis B Virus Ribonuclease H

John Tavis, Ph.D., *Saint Louis University School of Medicine, Saint Louis, MO, United States*
Razia Tajwar, Ph.D., *Saint Louis University School of Medicine, MO, United States*

Improved treatments for hepatitis B virus (HBV) are ugently needed. HBV replicates via reverse transcription catalyzed by the viral polymerase (P) with reverse transcriptase (RT) and ribonuclease H (RNase H) activities. Production of recombinant P and its domains is very challenging due to insolubility. Recently, we predicted and validated the first structure of P. We hypothesized HBV RNase H expression difficulties were because D788 (the last residue in the catalytic DEDD motif) resides in α-helix E in most RNases H, but is in an unstructured loop in the HBV RNase H. We mutated proline residues in "α-helix E" of HBV RNase H to P781F, P786E, P790L, P790L/786E, P790L/P781F, DPS789-791RLA, and P786+DPS789-791RLA. These replacements were based residues in human RNase H1 at positions of prolines in the HBV RNase H. Predicted mutant structures showed a more structured α-helix E compared to wildtype RNase H. Active enzyme was obtained for 100% of preparation for mutant enzymes compared to <10% for the wildtype. All mutations improved solubility and activity of the enzyme when expressed in E. coli. HBV RNase H inhibitors in the α-hydroxytropolone, N-hydroxypyridinedione, and N-hydroxynapthyridinone chemotypes docked into the predicted mutant structures with scores of -6.4 to -9.7 kCal/mol, with poses and scores being very similar to the wildtype enzyme. These data are being correlated with HBV replication inhibition data and RNase H inhibition data are being gathered. This work will enable mechanistic studies with the HBV RNase H and high throughput inhibitor screening against the HBV RNase H.

411. The Combination of Bemnifosbuvir (BEM) and Ruzasvir (RZR), the HCV NS5b and NS5A Inhibitors, Demonstrates Potent In Vitro Synergistic Antiviral Activity and In Vivo Preclinical Safety Without Adverse Precautions

Nancy Agrawal, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Qi Huang, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Kai Lin, Ph.D., *Atea Pharmaceuticals, Inc., Massachusetts, United States*
Shouqi Luo, Ph.D., *Former Atea employee, Boston, Massachusetts, United States*
Jean-Pierre Sommadossi, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*

BEM, the orally available prodrug of a GTP analog, has demonstrated highly potent and selective pangenotypic in vitro activity and best-in-class clinical efficacy against all HCV genotypes tested. The combination of this NS5B inhibitor with the potent, pangenotypic NS5A inhibitor RZR is being developed for improved treatment of HCV infections. The antiviral effects of 9 two-fold dilutions of BEM combined with 5 two-fold dilutions of RZR, each starting at twice their EC50 values, were determined in triplicate in HCV GT1b Huh-7 replicons. Analysis of two independent evaluations by Pritchard and Shipman MacSynergy II software provided synergy volumes at the 95% confidence interval (103 and 255 μM2%) that exceeded the 100 μM2% limit indicative of highly synergistic antiviral activity. Antagonistic or synergistic cytotoxicity was not observed. In a GLP-compliant rat toxicity study, BEM and RZR were orally administered independently and in combination at 500 mg/kg once daily for 13 weeks. All treatments were well tolerated and this dose was determined to be a no observable adverse effect level for both sexes. Systemic exposures of BEM, its metabolites, and RZR were similar when dosed alone or in combination, suggesting no drug-drug interactions (DDIs) that affected their pharmacokinetics even at this high dose. Given the highly potent pangenotypic activities of BEM and RZR, their complementary mechanisms of action, their clinically demonstrated safety and efficacy and their tolerability and lack of DDIs in rats at a dose well above therapeutic levels, combination treatment of HCV infections with these two agents is highly attractive and is being evaluated in clinical trials.

412. Within-cell Dynamics of Wildtypem Virus-defective Genomes-RNA Satellites: A Mathematical Approach

Josep Sardanyés, Ph.D., *Centre de Recerca Matemàtica, Cerdanyola del Vallès, Barcelona, Spain*
José Tomás Lázaro, Ph.D., *Universitat Politècnica de Catalunya, Barcelona, Spain*
Santiago Elena, Ph.D., *Institute for Integrative Systems Biology (I2SysBio), València, Spain*
Ariadna Albó, M.S., *Spain*
Tomás Alarcón, Ph.D., *Centre de Recerca Matemàtivca, Barcelona, Spain*

Defective viral genomes are naturally synthesized by almost all viruses. It was early identified that such deleting genomes can interfere with the regular replication of the standard viral genomes and multitude of experimental and clinical essays have revealed their presence. The dynamics of interference of these viral defective interfering particles (DIPs) has been largely studied with mathematical models at different biological scales, including within- cell, within-host, and population levels. Despite this intensive research, the interaction between DIPs and viruses carrying a satellite remains unexplored. Satellites are viruses that parasitize other viruses and that must co-infect with the wildtype (wt) to complete their reproduction cycle. Here, we investigate a simple mathematical model describing the dynamics between a wt virus generating DIPs and replicating under the presence of a satellite. Our model, as far as we know, is the first attempt to describe the dynamics of this 3-virus system. The model includes the processes of viral complementation, competition and different interference strengths by DIPs and the satellite on the wt virus. We have computed the equilibrium points, providing the conditions for the extinction and coexistence of the populations. We have identified transcritical bifurcations involving the transition from a satellite-free scenario i.e., coexistence standard virus-DIPS, to the extinction of the three viral types. Our analytic and numerical results focus on the stability of the system giving place to different scenarios which are relevant from a biomedical point of view.

413V. Novel pyrrolopyrimidine Nucleoside Analogs As Anti-hepatitis B Virus Agent

Omar Moukha-Chafiq, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Larry Bratton, M.S., *Southern Research, United States*
Mark Suto, Ph.D., *United States*
Corinne E. Augelli-Szafran, Ph.D., *Southern Research, Birmingham, AL, United States*

Hepatitis B virus (HBV) infection is one of the most common infectious diseases in the world. Chronic hepatitis B represents a critical unmet medical need with over 240 million people chronically infected worldwide. Over the last twenty years, significant progress has been made to develop approaches to control HBV infections and to prevent the development of hepatocellular carcinoma using various interferons and small molecules as antiviral agents. However, none of these agents had significant impact on eliminating HBV from infected cells. Through our medicinal chemistry efforts, we have synthesized and evaluated the anti-HBV activity of several pyrrolo[3,2-d]pyrimidine nucleoside analogs using HepG2 cells. Amongst these analogs, SRI-31416 showed potent antiviral activity with an EC50 of 70 nM against HBV and no observed cytotoxicity up to 10 μM concentration. SRI-31416 has acceptable in vitro solubility (76 μM), microsomal stability in both mouse and human species (half-life t1/2 > 3 hours) as well as reasonable mouse in vivo PK properties. Based on pre-clinical in vitro data, SRI-31416 will be tested in an adeno-associated vector-based HBV mouse model.

414V. Systematic Mutagenesis Studies Reveal Amino Acid Residues Critical for HBeAg Biogenesis and Mechanism of Capsid Assembly Modulator Inhibition of HBeAg Secretion

Hui Liu, Ph.D., *Baruch S Blumberg institute, Doylestown, Pennsylvania, United States*
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, PA, United States*
Ju-Tao Guo, M.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

Hepatitis B virus precore protein (p25) shares the entire core protein (Cp) polypeptide with an additional 29 amino acid (aa) residues at its N-terminus. Cleavage of the N-terminal 19 aa signal peptide in the endoplasmic reticulum produces p22, which is subsequently transported to the Golgi complex where the C-terminal arginine-rich domain is cleaved by furins to yield p17 and secreted out as homodimers, i.e., HBeAg, a putative immune suppressor contributing to the persistence of HBV infection. Our recent study showed that mutations of amino acid residues at Cp dimer-dimer interface, such as P25A, T33N and I105F, not only conferred resistance to capsid assembly modulator (CAM) disruption of capsid assembly, but also inhibition of HBeAg secretion, suggesting that CAMs may target the similar structures at "HAP" pocket between the homodimers of Cp or p17 to misdirect their assembly (Hui Liu, et al. PLoS Pathogens, 2021). However, selected capsid assembly deficient Cp mutations, W102A, G123A and Y132A, in the context of p25 showed variable responses to CAMs, indicating additional interaction between CAMs and p17 dimers may also attribute to the inhibition of HBeAg biogenesis. Studies are currently under the way to identify additional key residues in p17 for CAM inhibition of HBeAg secretion with a panel of mutant p25 proteins spanning the entire Cp assembly domain and in vitro p17 assembly assays. Better understanding the MOA of CAM inhibition of HBeAg biogenesis allows the discovery of antivirals for efficient suppression of HBeAg production, which may facilitate the restoration of host antiviral immune response and achievement of functional cure of chronic hepatitis B.

500. 27-hydroxycholesterol Inhibits Rhinovirus Replication in vitro and On Human Nasal and Bronchial Histocultures Without Selecting Viral Resistant Variants

Matteo Costantino, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*
Andrea Civra, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*
Roberta Cavalli, Ph.D., *Department of Drug Science and Technology, University of Turin, Torino, Italy*
Marco Adami, *Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy*
Marco Volante, *Department of Oncology, University of Turin, University of Turin, Orbassano, Torino, Italy*
Giuseppe Poli, *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*
David Lembo, Ph.D., *Department of Clinical and Biological Sciences, University of Turin, Orbassano, Torino, Italy*

Viral quasispecies are defined as a group of genetically variegated yet closely related viruses subjected to a constant process of genetic variation, competition, and selection. The genetic variability of these pathogens represents a hard challenge for the development of direct-acting antivirals (DAAs), since they exert selective pressure on the viral population, thereby leading to inhibitor-resistant strains. It is generally accepted that host-targeting antivirals (HTAs) can overcome this mechanism and are likely to generate a lower number of resistant variants if compared to DAAs. The aim of this study is to provide empirical proof-of-principle of the actual greater genetic barrier of 25-hydroxycholesterol (25OHC) and 27-hydroxycholesterol (27OHC), two physiologic oxysterols and HTAs, using rhinovirus (HRV) as a quasispecies model. We demonstrated that neither 25OHC nor 27OHC select oxysterols-resistant HRV variants by means of clonal or serial passages approaches. The antiviral potential of these oxysterols was confirmed during in vitro antiviral assays, where they demonstrated strong inhibition of both HRVA1 and HRVB48 replication and viral progeny. Ultimately, we selected 27OHC to further confirm its anti-HRV efficacy on 3D in vitro fully reconstituted human respiratory epithelia derived from cystic fibrosis patients, demonstrating its protective effect against tissue damage and cilia destructions. Taken together, these data suggest that 27OHC antiviral potential should be considered further and provide a rationale for studies aiming at exploring its potential for preclinical development.

501. A Broad-spectrum Small Molecule Anti-viral Targeting SARS-CoV-2 and Pandemic Influenza Viruses.

Gudepalya Renukaiah Rudramurthy, Ph.D., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Chandra Sekhar Gudla, Ph.D., *Foundation For Neglected Disease Research, Karnataka, India*
Rajappa Harish, M.S., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Yogi Maddipatla, M.S., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Naveen Kumar Nanjegowda, M.S., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Bharath Kumar Kumara Swamy, M.S., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Siva Shanmugam, M. Pharm, *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Mayas Singh, M.S., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Radha Krishan Shandil, Ph.D., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*
Shridhar Narayanan, Ph.D., *Foundation For Neglected Disease Research, Bengaluru, Karnataka, India*

The COVID-19 pandemic led to millions of deaths globally and influenza has been estimated to cause 290000 to 650000 deaths per year globally. Currently, vaccination remains the most effective way to counteract COVID-19 and influenza, and only few antiviral medications are available. The constant evolution and development of antiviral resistance by SARS-CoV-2 and influenza viruses is a great concern. Hence, there is an unmet medical need for developing broad-spectrum antiviral agents. We have recently, discovered a broad-spectrum antiviral small molecule inhibitor, FNDR-11124. The FNDR-11124 is a novel, synthetic flavonoid compound with broad spectrum in-vitro antiviral activity against SARS-CoV-2 and Influenza A (H1N1) and B, including subtypes of influenza A (H5N1, H2N3 and H7N9) with IC50 ranging from 0.5 μM to 2.0 μM. FNDR-11124 is orally bioavailable and has a suitable PK and DM profile to progress into efficacy studies. In vivo studies in the Syrian golden hamsters' infection model for SARS-CoV-2 has shown significant reduction (~1.0 Log10 TCID50/Lung) in lung viral load, and reduced virus induced lung pathology. Similarly, FNDR-11124 resulted in 100% survival of BALB/c mice infected with lethal dose of influenza (H1N1 A/Puerto Rico/8/34 (PR8) in both short term (7 day) and long term (21 day) models associated with significant reduction in lung viral loads (>1.0 Log10 TCID50/Lung), and reduced virus induced lung pathology. Our preliminary

data show that, FNDR-11124 is a promising early lead compound around which a chemistry effort can be made to deliver a broad-spectrum therapeutic molecule against SARS-CoV-2, influenza, and possibly, other respiratory viruses.

502. A High-throughput Discovery Platform Supporting Early and Advanced Antiviral Programs

Miranda Nebane, Ph.D., *Southern Research, Birmingham, AL, United States*
Lynn Rasmussen, M.S., *Southern Research, AL, United States*
Yohanka Martinez-Gzegozewska, M.S., *Southern Research, Birmingham, AL, United States*
Shalisa Sanders, *Southern Research, Birmingham, AL, United States*
Andrew Reece, *Southern Research, Birmingham, AL, United States*
Ling Zhai, *Southern Research, Birmingham, AL, United States*
Sara Cabrera, *Southern Research, Birmingham, AL, United States*
Melinda Sosa, *Southern Research, Birmingham, AL, United States*
Anna Manuvakhova, *Southern Research, Birmingham, AL, United States*
Pedro Ruiz, *Southern Research, Birmingham, AL, United States*
Omar Moukha-Chafiq, Ph.D., *Southern Research, Birmingham, AL, United States*
Stephanie Moore, Ph.D., *University of AL at Birmingham, Birmingham, AL, United States*
Richard J. Whitley, M.D., *University of AL at Birmingham, Birmingham, AL, United States*
Corinne E. Augelli-Szafran, Ph.D., *Southern Research, Birmingham, AL, United States*
Paige Vinson, Ph.D., *Southern Research, Birmingham, AL, United States*

The consequences of a global pandemic have been made a reality over the past three years. This event prompted the resurgence of new antiviral and vaccine programs which demonstrated that significant advances can occur in short periods of time with the appropriate tools, methods, and resources. The generation of large numbers of samples, whether small molecules from a discovery program or samples from a clinical trial, necessitates a high-throughput approach to provide timely results for decision-making and next steps. We have established a platform consisting of cell-based assays in 384-well format and multiple modes of detection, including cytopathic effect (CPE), immunofluorescence (IF), AlphaScreen (Alpha), and reporter viruses. Assays for different virus strains within the families of Coronavirus, Influenza, Alphavirus, Flavivirus, and Pneumovirus have been developed and are available for rapid turnaround of results. The development process has involved selection of appropriate cell lines and detection modes, and determination of optimal virus addition. The optimized assays exhibit a reproducible Z' value > 0.5 and have been confirmed relevant by measuring the activity of known active reference small molecules or antibodies. When required, correlation of results to other established assays have been obtained to validate the high-throughput approach. The establishment of this platform has generated over two million data points, enabling the progress of multiple programs in various stages of drug discovery. The general development process, challenges encountered, and representative results for each of the assay formats highlighted will be presented and discussed.

503. A Mutation in the Coronavirus nsp13-Helicase Confers Partial Remdesivir Resistance and Alters Enzymatic Activity

Mark Denison, M.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Samantha Grimes, Ph.D. Seeking, *Vanderbilt University Medical Center, TN, United States*
Young Choi, B.S., *The Rockefeller University, New York, NY, United States*
Anoosha Banerjee, Ph.D. Seeking, *The Rockefeller University, New York, NY, United States*
Gabriel Small, Ph.D. Seeking, *The Rockefeller University, New York, NY, United States*
Jordan Anderson-Daniels, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Jennifer Gribble, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Andrea Pruijssers, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Maria Agostini, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Seth Darst, Ph.D., *The Rockefeller University, New York, NY, United States*
Elizabeth Campbell, Ph.D., *The Rockefeller University, New York, NY, United States*

Remdesivir (RDV) is an FDA-approved antiviral in use to treat SARS-CoV-2 (COVID) infections. In coronaviruses (CoVs), RDV resistance mutations have been reported in the CoV nonstructural protein 12 RNA-dependent RNA polymerase. Here, we report that a previously identified RDV-selected mutation in the CoV nsp13-helicase (nsp13-HEL A335V) of

the coronavirus murine hepatitis virus (MHV) confers partial RDV resistance, which is both independent and additive when combined with previously reported nsp12-RdRp substitutions. The nsp13 A335V mutant is has decreased fitness compared to wild-type MHV and does not demonstrate cross-resistance to the nucleoside analog antiviral molnupiravir. Biochemical analysis of the SARS-CoV-2 nsp13-helicase demonstrated that substitution at the conserved residue (A336V), while still allowing association of nsp13 with the nsp 7-8(2)-12 core replication complex, has impaired helicase unwinding and ATPase activity. Finally, the SARS-CoV-2 nsp13-A336V is naturally occurring in SARS-CoV-2 isolates reported on the GISAID database. Together, these data define a potential novel pathway for RDV resistance, highlight a novel determinant of helicase enzymatic activity, and support surveillance for and testing of helicase mutations that arise in SARS-CoV-2 genomes.

504. A Non-Excisable Nucleotide Analogue Active against SARS-CoV-2

Ashleigh Shannon, Ph.D., *AMU, Marseille, France*
Aurélie Chazot, *AFMB, CNRS, Aix-Marseille University, UMR 7257, France*
Camille Falcou, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*
Veronique Fattorini, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Adel Moussa, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Steven Good, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Jean-Pierre Sommadossi, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
François Ferron, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France*
Bruno Canard, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*

For Coronaviruses, NA potency is compromised by natural resistance provided by association of the RdRp with the viral 3-to-5′ exonuclease nsp14/nsp10. This proofreading and repair complex removes terminal analogues from viral RNA, compromising the potency of several known NAs such as e.g., Ribavirin, 5-FU, and Remdesivir.

Phosphodiester bonds carrying a non-bridging sulfur atom (thiophosphates) are known to inhibit several nucleic acid exonucleases, but nothing is known in the case of nsp14/nsp10. Before being excised (or not), they would have to be activated and incorporated into viral RNA by the viral RdRp. Alas, the activation of α-thio NAs to their 5′-TP-α-thio form in unknown, neither is known if these 5′-TP- NA α-thiophosphate would be substrates for the SARS-CoV-2 RTC, not to mention which isomer Rp or Sp would be preferred.

Bemnifosbuvir (AT-527) is a NA in clinical trials phase III against SARS-CoV-2. We synthesized AT-1000, related to Bemnifosbuvir but bearing a sulfur atom on its α-phosphate (ie, α-thio). It exhibits potent anti-SARS-CoV2 activity (EC90=0.15 μM in HAE cells), comparable to Bemnifosbuvir.

Here we show that it is activated by cellular kinases hGUK1 and hNDPK up to the 5′-TP Sp AT-9052 preferentially. Remarkably, only the Sp isomer both causes immediate chain-termination and is completely resistant to nsp14-mediated excision. More remarkably, neither the Sp nor Rp isomer inhibits the NiRAN domain nucleotidylation activity. The α-thio modification therefore creates a novel drug, exhibiting an original and general mode of action against RNA viruses carrying natural resistance, such as highly pathogenic coronaviruses.

505. A Novel Transgenic Mouse Model (R26AGP-hACE2 in B6 Mice) Expressing Human ACE2 for Studying the Pathogenesis of SARS-COV-2 Infection

An-Yu Chen, M.S., *The Institute of Preventive Medicine, National Defense Medical Center, Taipei, Taiwan, Republic of China*
You-Tzung Chen, *Taiwan, Republic of China*
I-Shing Yu, *Taiwan, Republic of China*
Chih-Heng Huang, *Taiwan, Republic of China*

Since the SARS-CoV-2 outbreak in 2019, over 623 million cases and 6.5 million deaths have been reported as of 21 Oct 2022. As virus variants and the consequent demand for drug discovery emerge, available animal models, grow essential for the evaluation of antiviral and vaccine candidates, as well as for the investigation of the pathogenesis and transmission of the pandemic disease. Currently, K18-hACE2 mice are the major representative mouse model of SARS-CoV-2 infection. However, the convenience and cost of sufficient quantity to obtain is still a problem in Taiwan. Here we show a novel hACE2 transgenic mouse (SOX2cre; R26AGP in B6 mice) of stably expressed hACE2 with a

high safety level and detectable expression by the GFP tracker as a model of SARS-CoV-2 infection. The SOX2cre; R26AGP infected mice generated typical interstitial pneumonia and pathology that were similar to those of K18-hACE2 infected mice. Moreover, SOX2cre; R26AGP mice showed higher susceptibility to SARS-CoV-2 than K18-hACE2 mice in terms of survival and weight change. Viral quantification by tittering with plaque assay and quantitative real-time PCR, which performed viral titer in SOX2cre; R26AGP mice was the same as K18-hACE2 mice and even earlier. SOX2cre; R26AGP mice are a successful SARS-CoV-2 hACE2 transgenic mouse infection model and can cut down the experimental timeline. Additionally, gastrointestinal (GI) symptoms in patients were seen in (about 22%) long COVID, We are going to establish the R26AGP mouse model combined with the small and large intestines tissue-specific cre model, Vil1-cre mice, for instance, to investigate further the pathogenesis of long COVID GI symptoms in vivo.

506. A Reporter Cell Line for the Automated Quantification of SARS-CoV-2 Infection in Living Cells

Yves Rouillé, Ph.D., *CNRS, Lille, France*
Lowiese Desmaets, *France*
Nathalie Callens, *France*
Eik Hoffmann, *France*
Adeline Danneels, *France*
Muriel Lavie, *France*
Cyril Couturier, *France*
Jean Dubuisson, *Center for Infection and Immunity of Lille (CIIL), France*
Sandrine Belouzard, *Center for Infection and Immunity of Lille (CIIL), France*

The SARS-CoV-2 pandemic and the urgent need for massive antiviral testing highlighted the lack of a good cell-based assay that allowed for a fast, automated screening of antivirals in high-throughput content with minimal handling requirements in a BSL-3 environment. The present paper describes the construction of a green fluorescent substrate that, upon cleavage by the SARS-CoV-2 main protease, re-localizes from the cytoplasm in non-infected cells to the nucleus in infected cells. The construction was stably expressed, together with a red fluorescent nuclear marker, in a highly susceptible clone derived from Vero-81 cells. With this fluorescent reporter cell line, named F1G-red, SARS-CoV-2 infection can be scored automatically in living cells by comparing the patterns of green and red fluorescence signals acquired by automated confocal microscopy in a 384-well plate format. We show the F1G-red system is sensitive to several SARS-CoV-2 variants of concern and that it can be used to assess antiviral activities of compounds in dose-response experiments. This high-throughput system will provide a reliable tool for antiviral screening against SARS-CoV-2.

507. Analysis of SARS-CoV-2 Variants From Patient Specimens in Nevada from October 2020 to August 2021

Cyprian C. Rossetto, *University of Nevada, Reno School of Medicine, Reno, NV, United States*

In early 2020, the emergence and spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the human population quickly developed into a global pandemic. SARS-CoV-2 is the etiological agent of coronavirus disease 2019 (COVID-19) which has a broad range of respiratory illnesses. As the virus circulates, it acquires nucleotide changes. These mutations are due to the inherent differences in the selection pressures within the human population compared to the original zoonotic reservoir of SARS-CoV-2 and formerly naïve humans. The acquired mutations will most likely be neutral, but some may have implications for viral transmission, disease severity and resistance to therapies or vaccines. The primary goals of the current study were to determine the phylogenetic relationship of the SARS-CoV-2 genomes within Nevada and to determine if there are any unusual variants within Nevada compared to the current database of SARS-CoV-2 sequences. Whole genome sequencing and analysis of SARS-CoV-2 from 425 positively identified nasopharyngeal/nasal swab specimens were performed from October 2020 to August 2021 to determine any variants that could result in potential escape from the therapeutics. Our analysis focused on nucleotide mutations that generated amino acid variations in the viral Spike (S) protein, Receptor binding domain (RBD) and the RNA-dependent RNA-polymerase (RdRp) complex. The data indicate that SARS-CoV-2 sequences from Nevada did not contain any unusual variants that had not been previously reported. The clade trends in Nevada closely mirror what was reported for the United States; this includes clade 21A (Delta) being the predominant clade by August 2021.

508. Anchimerically Activatable ProTide Inhibitors of Eukaryotic Translation Initiation Factor 4E (eIF4E) as Host-Directed Antivirals Against SARS-CoV-2

Jacob A Smith, Ph.D. Seeking, *University of Minnesota-Twin Cities, Minneapolis, MN, United States*
Romario Matos de Souza, Ph.D., *Universidade Federal do Rio de Janeiro, Brazil*
Carston Rick Wagner, Ph.D., *University of Minnesota-Twin Cities, Minneapolis, MN, United States*
Luciana Jesus da Costa, Ph.D., *Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*

Currently, only a few small molecule therapeutics are available to treat SARS-CoV-2 infections and those that exist have significant limitations. Remdesivir (Veklury®) is fully FDA-approved in the United States for the treatment of COVID-19, but its clinical utility is severely limited by its cost, narrow therapeutic window, and the need for it to be administered intravenously. Molnupiravir and Paxlovid have been granted emergency use authorization in the United States but have limiting safety profiles. Additionally, host-directed antivirals that target endogenous proteins essential to viral replication pose a lower risk for driving mutations and are expected to retain their potency against emerging variants. Recently, eukaryotic translation inhibitors have emerged as exceptionally potent host-directed antivirals against SARS-CoV-2. Among these, plitidepsin and zotatifin have entered clinical trials for the treatment of moderate COVID-19. Plitidepsin inhibits eukaryotic translation elongation factor 1A (eEF1A) while zotatifin targets the RNA helicase eukaryotic translation initiation factor 4A (eIF4A). Inspired by the promising pre-clinical data of these translation inhibitors, we tested our previously developed eukaryotic translation initiation factor 4E (eIF4E) inhibitors, designated as 4Ei-10 and 4Ei-11, for their effect on SARS-CoV-2 replication. We observed significant replication inhibition at nanomolar concentrations in our initial in vitro viral plaque assays, as well as a significant reduction in viral spike protein expression. We anticipate that targeting eIF4E will represent a new avenue for therapeutic development in the treatment of COVID-19.

509. Antiviral Activity of a Metabolite from Hypericum Perforatum L. Against Human Coronaviruses

Imelda Raczkiewicz, M.S., *Center for Infection and Immunity of Lille (CIIL), Lille, France*
Lowiese Desmarets, Ph.D., *Center for Infection and Immunity of Lille (CIIL), France*
Gabriel Lefèvre, *UMR-T 1158 BioEcoAgro Univ Lille, France*
Nathan François, *Center for Infection and Immunity of Lille (CIIL), France*
Peggy Bouquet, *Center for Infection and Immunity of Lille (CIIL), France*
Jennifer Samaillie, *UMR-T 1158 BioEcoAgro Univ Lille, France*
Sevser Sahpaz, *UMR-T 1158 BioEcoAgro Univ Lille, France*
Jean Dubuisson, *Center for Infection and Immunity of Lille (CIIL), France*
Sandrine Belouzard, *Center for Infection and Immunity of Lille (CIIL), France*
Anne Goffard, *Center for Infection and Immunity of Lille (CIIL), France*
Céline Rivière, *UMR-T 1158 BioEcoAgro Univ Lille, France*
Karin Séron, *Center for Infection and Immunity of Lille (CIIL), France*

AIM: The pandemic of SARS-CoV-2 has highlighted the urge of broad-spectrum antivirals. Around 80% of the population relies on natural compounds to heal themselves, as they are a source of active molecules. We have screen different plant extracts against HCoV-229E and identified Hypericum perforatum L. (perforate St John's wort) as potentially active against human coronaviruses (HCoV). This plant is well known for its depressant properties. The aim is to study the antiviral activity of one of its main metabolites against HCoV and determine its mechanism of action.

METHODS: Dose-response experiments were performed on HCoV-229E-Rluc and SARS-CoV-2 in Huh-7 expressing TMPRSS2 and Vero81 expressing TMPRSS2 cells respectively by adding increasing concentrations of MM. The results were analyzed by measuring the luciferase activity or by virus titration (TCID50). Time-addition assays were done by adding MM before, during or after inoculation of the virus at the concentration of IC90. Cytotoxicity was determined by MTS assay.

RESULTS: We showed that MM exhibits a dose dependent activity on HCoV-229E (IC50: 1,12 µM), and on SARS-CoV-2 (IC50: 0,24 µM) without any cytotoxicity at those concentrations (CC50 > 60µM). These data were confirmed in a pulmonary cell line (A549 cells expressing ACE2 receptor). Time-addition experiments indicate that MM is active at a later stage of the virus cycle: either translation or replication.

CONCLUSION: Our data show for the first time that MM is highly active on HCoV. We are currently performing tests on primary respiratory epithelial cells to confirm our results. Experiments are underway to better characterize its mechanism of action.

510. Antiviral Activity of EIDD-1931 and Remdesivir Against SARS-CoV-2 Variants in 3D Mucociliary Tissue Models Consisting of Normal, Human-derived tracheal/Bronchial (EpiAirway) and Nasal (EpiNasal) Epithelial Cells

Kie Hoon Jung, Ph.D., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States*
Jonna B. Westover, Ph.D., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Utah, United States*
James K. Hansen, B.S., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States*
Inioska Rojas, B.S., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States*
Jung-Ae Choi, M.S., *Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan, Utah, United States*
Brett L. Hurst, Ph.D., *Utah State University, Logan, Utah, United States*

In vitro assays are an important early step to determine the potential antiviral activity of novel compounds. The antiviral efficacy of compounds is often evaluated in immortalized cell lines, which offer the advantage of cost effectiveness, ease of use, and reproducibility. However, these monolayer cell lines are not always predictive for cytotoxicity or in vivo efficacy. The EpiAirway™ and EpiNasal™ (MatTek) 3D mucociliary tissue models consisting of normal, human-derived tracheal/bronchial or nasal epithelial cells provide human-relevant tissue models for respiratory disease and antiviral research. We have developed advanced antiviral assays to evaluate promising compounds against SARS-CoV-2, MERS-CoV and other human respiratory pathogens by completing virus replication curves and evaluating compound cytotoxicity with the EpiAirway™ and EpiNasal™ models. Remdesivir and EIDD-1931 were shown to significantly reduce viral replication against several strains of SARS-CoV-2, MERS-CoV and other human respiratory viruses. Additionally, these 3D tissue models were more sensitive for detecting antiviral efficacy and less sensitive to compound toxicity compared to commonly used monolayer cell lines. The EpiAirway™ and EpiNasal™ human tissue models are a valuable tool for evaluating antiviral compounds prior to advancement to in vivo models for respiratory diseases. Future antiviral assay development will include oral, vaginal, spleen, liver, and small intestine tissue models of human diseases which will allow evaluation of additional non-respiratory viruses.

511. Antiviral Development of Pan-coronavirus Small Molecules Targeting Intracellular Dynamics

Raphael Gaudin, Ph.D., *CNRS, Montpellier, France*
William Bakhache, Ph.D., *France*
Yonis Bare, Ph.D., *France*
Gaia Cogrossi, *France*
Emma Partiot, *France*
Vincent Lucansky, Ph.D., *Slovakia*
Boris Bonaventure, Ph.D., *France*
Caroline Goujon, Ph.D., *France*
Maika Deffieu, Ph.D., *France*

SARS-CoV-2 (CoV2) is the viral agent responsible for the pandemic of the coronavirus disease 2019 (COVID-19). Vaccines are being deployed all over the world with good efficacy, but potent antiviral treatments are needed, because of the short-lasting effect of vaccination and the emergence of variants of interest. Here, we developed a series of small molecules and identified RG10 and several derivatives showing potent antiviral activity against the alpha-coronavirus HCoV-229E and the beta coronaviruses SARS-CoV-2 and MHV in cell lines with EC50 around 1 μM. RG10 localizes to endoplasmic reticulum (ER) membranes, perturbing ER morphology and inducing ER stress. Yet, RG10 does not associate with viral replication sites although preventing virus replication. To further investigate the antiviral properties of

our compound, we developed fluorescent SARS-CoV-2 viral particles allowing us to track virus arrival to ER membranes. Live cell imaging of replication-competent virus infection revealed that RG10 stalls the intracellular virus-ER dynamics. Finally, we synthesized RG10 derivatives and identified several compounds with increased potency in vitro and in primary human airway epithelia (HAE) and a pharmacokinetic half-life greater than 2 h in mice. Although the specific target of RG10 is unknown, we are undergoing original proteomic-based analyses to investigate the target/pathway conferring antiviral activity to RG10. Together, our work reports on a novel fluorescent virus model and innovative antiviral strategy consisting of the perturbation of ER/virus dynamics, highlighting the promising antiviral properties of RG10 and derivatives.

512. Antiviral Effect of 2-Deoxy-D-Glucose on Replication of Human Coronaviruses

Vitalii Kovtunyk, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Nicole Doyle, Ph.D., *The Pirbright Institute, United Kingdom*
Laxmikant Wali, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Michael Karbiener, Ph.D., *Takeda Manufacturing Austria AG, Vienna, Austria*
Scharon Chou, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Delyana Stoeva, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Thomas R. Kreil, Ph.D., *Takeda Manufacturing Austria AG, Vienna, Austria*
Johannes Stöckl, Ph.D., *G.ST Antivirals GmbH, Vienna, Austria*
Guido A. Gualdoni, M.D., Ph.D., *G.ST Antivirals GmbH, Vienna, Austria*
Helena Maier, Ph.D., *The Pirbright Institute, Woking, United Kingdom*
Anna-Dorothea Gorki, Ph.D., *Austria*

Common cold continues to be a great burden because of human suffering and high economic losses. Up to one third of patients with virus-induced common cold have detectable evidence of endemic human coronavirus (HCoV) infection with strains 229E, NL63 or OC43. It is known that such infections can lead to fatal outcomes in immunocompromised patients due to lower respiratory tract complications.

2-Deoxy-D-glucose (2-DG), a glucose analogue, inhibits glycolysis in infected cells and thereby targets the infection process within the cell rather than tackling the virus itself. We have observed significant decrease of released virus in samples treated with 2-DG and infected with endemic HCoV-229E, HCoV-NL63 or pandemic SARS-CoV-2 coronavirus. We further investigated the mechanisms of 2-DG antiviral effect on HCoV-229E using confocal microscopy and image quantification. We detected lower numbers of infected cells and viral double-stranded RNA foci in 2-DG-treated samples compared to mock-treated controls. Samples that were treated with 2-DG also had significantly lower number of cells with actively replicating viral RNA and lower numbers of active viral RNA replication sites within the cells, as detected via 5'-bromouridine incorporation into growing RNA chains. The obtained results provide insights into the mode of action of 2-DG against endemic HCoV-229E and the pathways it disrupts, such as viral RNA replication. The broad-spectrum antiviral activity of 2-DG demonstrates the potential for its use as an antiviral agent, as well as for development of new metabolic analogues targeting intracellular processes critical for virus infection.

513. Antiviral Properties of Stilbene Dimers Obtained Bychemoenzymatic Synthesis Against Enveloped Viruses

Arnaud Charles-Antoine Zwygart, M.S., *University of Geneva, Geneva, Switzerland*

Over the last century, the number of epidemics caused by RNA viruses has raised consistently and the current SARS-CoV-2 pandemic has taught us about the compelling need for ready to use broad-spectrum antivirals. In this scenario, natural products stand out as a major historical source of drugs. We analyzed the antiviral eff ect of new stilbene dimers obtained from plant substrates using chemoenzymatic synthesis against a panel of enveloped viruses. We report that our compounds display a broad-spectrum antiviral activity against enveloped viruses, being able to eff ectively inhibit several strains of Infl uenza viruses, as well as SARS-CoV-2. They are eff ective when administered before the infection, to mimic preventive treatments or after, to simulate therapeutic interventions. We used both in vitro and ex vivo models of infection, the latter being more clinically relevant, to corroborate our results combining diff erent methodological approaches. Interestingly, stilbene dimers exert antiviral activity to diff erent extents against diff erent viral species and with diff erent mechanisms of actions. Our fi ndings suggest a possible clinical application of stilbene dimers to the treatment of infections caused by enveloped viruses.

514. Apixaban, an Orally Available Anticoagulant, Inhibits SARS-CoV-2 Replication and its Major Protease in a Non-Competitive Way

Otavio Augusto Chaves, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*
Carolina Queiroz Sacramento, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*
Natalia Fintelman-Rodrigues, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*
Jairo Ramos Temerozo, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*
Filipe Pereira-Dutra, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil*
Daniella M. Mizurini, Ph.D. Seeking, *Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil*
Robson Q. Monteiro, Ph.D., *Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil*
Leonardo Vazquez, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil*
Patricia T. Bozza, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil*
Hugo Caire Castro-Faria-Neto, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil*
Thiago Moreno L. Souza, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*

Anticoagulant administration is associated with clinical benefits in 2019 coronavirus disease (COVID-19) patients mainly to prevent coagulopathy. Considering that clotting factor Xa, thrombin, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) main protease (Mpro) share over 80% homology at the three-dimensional protein level, it is worth interrogating whether there is crosstalk between substrates and inhibitors between these enzymes. If so, chemical structures could be promising to further be used as antivirals and anticoagulants. We found that apixaban, rivaroxaban, and dabigatran inhibited SARS-CoV-2 Mpro, in special, apixaban was 15-fold more potent than the other anticlotting (non-competitive mechanism). In silico results suggested that apixaban interacts with the enzyme-substrate complex and allosteric site of protease. Experimental clotting chromogenic substrates for thrombin (S-2238) and factor Xa (S-2765) were neither converted into a product by PLpro nor Mpro. The results were validated in pneumonocyte type II cells (Calu-3), where apixaban showed the most promisor potency (1.85 ± 0.09 uM).
Under clinically approved posology of 10 mg, apixaban reaches a maximum plasmatic concentration (Cmax) of 0.55 mM, with a free fraction at Cmax of 72 nM, almost 10-fold higher than apixaban's Ki toward Mpro. Additionally, apixaban's potency against SARS-CoV-2 in vitro replication was a multiplicity of infection (MOI)-dependent, ranging from lower to three times higher than human Cmax. Therefore, we consider the apixaban chemical structure as a lead to be optimized for the development of novel non-competitive Mpro inhibitors that preserve anticoagulant activity

515. Biochemical Characterization of Peptide-based Inhibitors of the Influenza Polymerase PA-PB1 Subunit Interaction

Michal Kral, Ph.D. Seeking, *Institute of Organic Chemistry and Biochemistry of the CAS, Prague, Czech Republic*

The influenza virus causes severe respiratory infection in humans and is responsible for 290-650 000 deaths annually. Despite the high prevalence of the virus, effective therapeutics are limited. Current antivirals target three key proteins in the viral life cycle: neuraminidase, the M2 channel and the endonuclease domain of RNA-dependent-RNA polymerase. Due to the development of novel pandemic strains, additional antiviral drugs targeting different viral proteins are still necessary. The protein-protein interaction between polymerase subunits PA and PB1 is one of possible targets. We recently identified a modified decapeptide derived from the N-terminus of the PB1 subunit with high affinity for the C-terminal part of the PA subunit. Here, we optimized its amino acid hotspots to maintain the inhibitory potency and

greatly increase peptide solubility. This allowed thermodynamic characterization of peptide binding to PA. Solving the X-ray structure of the peptide-PA complex provided structural insights into the interaction. Additionally, we optimized intracellular delivery of the peptide using a bicyclic strategy that led to improved inhibition in cell-based assays.

516. C6-Alkynyl-2,4-quinazolinedione-N-1-ribonucleoside Analogs and Their Phosphoramidates for the Inhibition of SARS-CoV-2.

Vincent Roy, Ph.D., *ICAO, Orléans, France*
Milène Franzetti, M.S., *University of Orléans (ICOA), Orléans, France*
El Hadji Cisse, *Centre de Biophysique Moléculaire, France*
Nicolas Biteau, *Emory University, Atlanta, United States*
Gaëlle Frenois-Veyrat, *Institut de Recherche Biomédicale des Armées, Brétigny-sur-Orge, France*
Marc Grandadam, *Institut de Recherche Biomédicale des Armées, Brétigny-sur-Orge, France*
Frédéric Iseni, *Institut de Recherche Biomédicale des Armées, Brétigny-sur-Orge, France*
Luigi Agrofoglio, Ph.D., *ICOA, Orléans, France*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly pathogenic positive-sense RNA virus responsible for the novel viral pandemic COVID-19. Beside the different vaccines and the only antiviral drug Paxlovid approved in the world, there is an urgent need for small molecules able to inhibit its viral replication. The viral RNA-dependent RNA polymerase (RdRp), key enzyme in the viral transcription and replication, is a major target of antiviral therapy, for which nucleos(t)ide analogs are at the forefront. They could be either repositioned against SARS-CoV-2 such as Remdesivir and Molnupiravir or newly developed.

We report herein the design and synthesis of series of hitherto unknown C6-alkynyl-2,4-quinazolinedione N-1-ribonucleoside analogs and their phosphoramidates, which bearing modifications on both the base and sugar moieties. The synthesis pathway involves three key-steps: (1) the introduction of the nucleobase under Vorbrüggen conditions, (2) the modification at C6 position of the quinazolinedione moiety through microwave-assisted Sonogashira Pd(0) cross-coupling reaction, and (3) the introduction of the phosphoramidate moiety at 5'-position in order to reach the prodrug form. All synthesized compounds have been evaluated for their antiviral activity against SARS-CoV-2 and some of them inhibit its replication (IC50) in micromolar range. Chemical synthesis and biological data will be presented.

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517. Characterization of Antibodies Against SARS-CoV-2 Capping Enzymes nsp10 and nsp14 and Their Use as a Tool in SARS-CoV-2 Research

Vladimira Horova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Barbora Landova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Martin Klima, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the coronavirus disease-19 pandemic. One of the key components of the coronavirus replication complex are the RNA methyltransferases (MTases), enzymes crucial for RNA cap formation (1). Structures of these MTases have become recently available (2-4), however, their biological characterization within the infected cells remains largely unclear. Here, we report generation and characterization of novel monoclonal antibodies directed against the SARS-CoV-2 non-structural protein nsp10, a subunit of both the 2'-O RNA and N7 MTase (5), and against nsp14 protein, the coronaviral N7-MTase. We confirm that novel antibodies successfully recognize both the denatured protein using western blotting and the native protein using immunocytochemistry. We also investigated the subcellular localization of the SARS-CoV-2 MTases in infected cells and we show that the methyltransferase machinery is localized mainly in vesicular structures in the perinuclear region of the infected cells, where the virus is replicated.

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518. Combination of the Parent Analogue of Remdesivir (GS-441524) and Molnupiravir Results in a Markedly Potent Antiviral Effect in SARS-CoV-2 Infected Syrian Hamsters

Rana Abdelnabi, Ph.D., *Rega Institute, KU Leuven, Leuven, Belgium*
Piet Maes, Ph.D., *KU Leuven, Belgium*
Steven De Jonghe, Ph.D., *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Birgit Weynand, Ph.D., *Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*

Remdesivir was the first drug to be approved for the treatment of severe COVID-19; followed by molnupiravir (another prodrug of a nucleoside analogue) and the protease inhibitor nirmatrelvir. Combination of antiviral drugs may result in improved potency and help to avoid or delay the development of resistant variants. We set out to explore the combined antiviral potency of GS-441524 (the parent nucleoside of remdesivir) and molnupiravir against SARS-CoV-2. In SARS-CoV-2 (BA.5) infected A549-Dual™ hACE2-TMPRSS2 cells, the combination resulted in an overall additive antiviral effect with a synergism at certain concentrations. Next, the combined effect was explored in Syrian hamsters infected with SARS-CoV-2 (Beta, B.1.351); treatment was started at the time of infection and continued twice daily for four consecutive days. At 4 day 4 post-infection, GS-441524 (50 mg/kg, oral BID) and molnupiravir (150 mg/kg, oral BID) as monotherapy reduced infectious viral loads by 0.5 and 1.6 log10, respectively, compared to the vehicle control. When GS-441524 (50 mg/kg, BID) and molnupiravir (150 mg/kg, BID) were combined, infectious virus was no longer detectable in the lungs of 7 out of 10 of the treated hamsters (4.0 log10 reduction) and titers in the other animals were reduced by ~2 log10. The combined antiviral activity of molnupiravir which acts by inducing lethal mutagenesis and GS-441524, which acts as a chain termination appears to be highly effective in reducing SARS-CoV-2 replication/infectivity. The unexpected potent antiviral effect of the combination warrants further exploration as a potential treatment for COVID-19.

519. Curcuminoid Analogues Suppress Influenza A Virus Replication and Expression of Pro-inflammatory Cytokines and Interferons in A549 Lung Epithelial Cells by Inhibiting Multiple Retinoic Acid-inducible Gene-I (RIG-I)-mediated Pathways

Kong Yen Liew, Ph.D. Seeking, *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
Hui-Yee Chee, Ph.D., *Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang Selangor, Malaysia*
Faridah Abas, Ph.D., *Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
Sze Wei Leong, Ph.D., *Natural Medicines and Products Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
Hanis Hazeera Harith, Ph.D., *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
Daud Ahmad Israf, Ph.D., *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
Chau Ling Tham, Ph.D., *Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

Curcumin, a natural compound from Curcuma longa, has excellent anti-inflammatory and antiviral effects; however, poor bioavailability hinders its development. We have previously shown that two novel curcuminoid analogues with higher stability and solubility, namely 2-benzoyl-6-(3,4-dihydroxybenzylidene)cyclohexen-1-ol (BDHBC) and 5-(3,4-dihydroxyphenyl)-3-hydroxy-1-(2-hydroxyphenyl)penta-2,4-dien-1-one (DHHPD), have better antiviral effects against rhinovirus. Thus, we further investigate their effects on influenza A virus (IAV) by assessing the gene levels of viral nucleoprotein (NP), cytokines, interferons (IFNs) and interferon-stimulated genes (ISGs) and relating them to IAV-activated signalling pathways. A549 cells were infected with influenza A/PR/8/34 and treated with non-cytotoxic concentrations of curcumin, BDHBC or DHHPD (5, 10 and 20μM). RT-qPCR was used to measure the gene expression levels, whereas Western blot was used to investigate the target signalling pathways. Like curcumin, DHHPD significantly reduced the gene expression of NP, IL-6, IL-8, IP-10, IFN-β and IFN-λ1 in a concentration-dependent manner. By contrast, inhibition was only observed for the highest concentration of BDHBC (20μM). Interestingly, curcumin and both analogues had no effect on the activation of ISGs, including IRF7 and the cytosolic RNA sensor RIG-I. Western blot analyses revealed that they inhibited the activation of multiple pathways downstream of RIG-I such as Akt, MAPK (p38 and Erk1/2) and NF-κB. These findings suggest that they suppress RIG-I-mediated pathways without modulating its expression. They may be potential drug leads for IAV which target host signalling pathways.

520. Design and Synthesis of trimeric Compounds as Fusion Inhibitors of Hemagglutinin of Influenza Virus

Sonia De Castro, Ph.D., *Instituto de Química Médica (CSIC), Madrid, Spain*
Alvaro De la Cruz, M.S., *Instituto de Química Médica (CSIC), Spain*
Tiziana Ginex, Ph.D., *Departament de Nutricio, Ciencies de l'Alimentacio I Gastronomia, Institut de Biomedicina (IBUB) and Institut de Quimica Teorica i Computacional (IQTCUB), Facultat de Farmacia i Ciencies de l'Alimentacio, Universitat de Barcelona, Barcelona, Spain*
Lieve Naesens, Ph.D., *Rega Institute for Medical Research, KU Leuven, University of Leuven, Leuven, Belgium*
Francisco Javier Luque, Ph.D., *Departament de Nutricio, Ciencies de l'Alimentacio I Gastronomia, Institut de Biomedicina (IBUB) and Institut de Quimica Teorica i Computacional (IQTCUB), Facultat de Farmacia i Ciencies de l'Alimentacio, Universitat de Barcelona, Barcelona, Spain*
Sonsoles Velázquez, Ph.D., *Instituto de Química Médica (CSIC), Madrid, Spain*
María José Camarasa, Ph.D., *Instituto de Química Médica, Spanish Research Council (CSIC), Madrid, Spain*

Infection by influenza A and B viruses result in 3-5 million serious cases each year. The recent outbreaks of highly pathogenic (re)emerging influenza viruses, the shortage of drugs in clinical use and the low effectiveness due to the global transmission of influenza virus resistant to these drugs, highlights the need of novel influenza therapeutics directed to new targets and/or novel mechanism of action.

The trimeric hemagglutinin (HA) glycoprotein plays a critical role in virus entry, making it a highly attractive therapeutic target. We have recently identified a unique class of N-benzyl-4,4-disubstituted piperidines as influenza A virus fusion inhibitors with specific activity against the H1N1 subtype. Mechanistic and computational studies with the prototype compound revealed that the inhibitory activity is mediated through binding to a so-far unexplored pocket in the HA2 subunit of HA close to the highly conserved fusion peptide. The proposed binding model reveals that this compound only binds to the fusion peptide of one of the HA monomeric fusion peptides.

We herein report our first steps towards the design and synthesis of innovative trimeric influenza virus fusion inhibitors by taking advantage of the three-fold symmetry of the HA homotrimer. The general structure of the proposed compounds contain a central scaffold decorated with three identical arms bearing aromatic recognition motifs to establish π-stacking interactions with the Phe9 of the fusion peptides and an amino group at the focal point to form a salt bridge with the Glu120. A variety of scaffolds, covalent linker groups and arms of appropriated lengths are explored.

521. Development of New Benzofuran Derivatives as STING Agonists with Antiviral Activity

Annalaura Paulis, *Università degli studi di Cagliari, Cagliari, Italy*
Pierre-Olivier Vidalain, *Centre International de Recherche en infectiologie, France*
Vincent Lotteau, *Centre International de Recherche en infectiologie, Lyon, France*
Alessia Onali, *Università degli Studi di Cagliari, Cagliari, Italy*
Simona Distinto, Ph.D., *CADD unit Department of Life and Environmental Sciences University of Cagliari, Cagliari, Italy*
Giovanna Delogu, *Università degli studi di Cagliari, Cagliari, Italy*
Enzo Tramontano, Ph.D., *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*

STING is a transmembrane protein localized in the endoplasmic reticulum involved in type I Interferons (INF-I) transcription. When cytosolic DNA is detected cyclic GMP-AMP (cGAMP) synthase produces 2'3' cGAMP , that binds STING triggering IFN Regulatory Factor 3 (IRF3) phosphorylation and dimerization, this complex is translocated into the nucleus where stimulates IFN-I transcription. Recent studies identified STING agonists with antiviral activity. For this reason, a selection of putative STING-ligands was tested to evaluate their ability to induce IFN-I and inhibit viral replication. By applying two rounds of similarity ligand-based virtual screenings a focused library of eleven benzofurans has been selected and subjected to biological test. A gene reporter assay in cells expressing exogenous STING (HEK293T) was used to investigate compounds' dependent IFN-b transcription in presence of a luciferase reporter gene driven by the human IFN-b promoter. Tested compounds induced IFN-b transcription, while they were not able to induce IFN-b transcription in presence of mutated and inactive STING suggesting a specific protein-ligand interaction. Since DNA damage can indirectly activate STING and parallel pathways inducing IFN response, we evaluated compound's mediated genotoxic effect, through p53 levels analysis, as well as dihydroorotate dehydrogenase inhibition showing no effects in both cases. The best hit compounds were tested for the antiviral activity in BEAS-2B cells against human coronavirus 229E: three of them showed antiviral activity whit an IC50 value in the μM range. Their binding mode has rationalized by mean of molecular docking experiments.

523. EDP-235, a Potent, Once-daily, Oral Antiviral, Demonstrates Excellent Penetration into SARS-CoV-2 Target Tissues, with the Potential for Mitigation of Viral Rebound in COVID-19 Patients

Indy Zang, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Li-Juan Jiang, Ph.D., *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Yang Li, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Lisha Xu, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Daniel Leonard, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Khanh Hoang, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Tim Greizer, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Shucha Zhang, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Caroline Kerr, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Meng Huang, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Jonathan Kibel, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Kelvin Chan, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Joshua Klaene, *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*
Yat Sun Or, Ph.D., *Enanta Pharmaceuticals, Inc., Watertown, MA, United States*

Up to 27% of COVID-19 patients experience viral rebound after Paxlovid (ritonavir-boosted nirmatrelvir) treatment. Several reports have suggested that viral rebound can occur if SARS-CoV-2 remains in parts of the body that Paxlovid cannot access. Herein, we report that EDP-235, a novel and potent SARS-CoV-2 3C-like protease inhibitor, demonstrates superior penetration into SARS-CoV-2 target tissues in human cells and preclinical species compared to nirmatrelvir.

Intracellular uptake of EDP-235 was tested side-by-side with nirmatrelvir in human cells. The ratios of intracellular to extracellular concentrations of EDP-235 were 8.7 in human lungs, 9.9 in cardiac myocytes, 11.3 in salivary glands, 18.0 in kidneys, and 33.6 in adipocytes. In contrast, nirmatrelvir had ratios of 0.6 to 1.2 in these human cells.

To determine the in vivo drug distribution into SARS-CoV-2 target tissues, rats were dosed orally with 25 mg/kg of EDP-235 or nirmatrelvir. EDP-235 showed favorable rat plasma exposure of 19.0 μg-hr/mL, whereas nirmatrelvir had a significantly lower rat plasma exposure of 4.9 μg-hr/mL. Consistent with in vitro observations, EDP-235 displayed excellent target tissue exposure in rats with tissue to plasma ratios of 4.1 in lungs, 4.7 in heart, 6.5 in salivary glands, 6.3 in kidneys, and 23.0 in adipose tissues, whereas nirmatrelvir had corresponding ratios of 0.8, 0.9, 1.0, 1.2 and 0.6 in those tissues.

Preferential target tissue distribution may enable EDP-235 to minimize viral rebound in COVID-19 patients if administered as an initial treatment. A Phase 2 clinical trial of EDP-235 for the treatment of COVID-19 started in Q4 2022.

524. EDP-235, an Oral, Once Daily, Ritonavir-Free, 3CL Protease Inhibitor for the Treatment of COVID-19: Results From Phase 1 Study in Healthy Subjects

Guy De La Rosa, M.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Kimberly Mills, Pharm.D., *Enanta Pharmaceuticals, MA, United States*
Christine Marotta, Pharm.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Xiaohui Luo, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Kent Melchiors, M.D., *ICON, Lenexa, KS, United States*
Alaa Ahmad, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Guy De La Rosa, M.D., *Enanta Pharmaceuticals, Watertown, MA, United States*

Despite vaccination efforts, COVID-19 persists as a significant global health challenge, with ongoing emergence of new variants. EDP-235 is a novel, potent, oral inhibitor of the SARS-CoV-2 3CL protease designed for the treatment of COVID-19. Here, we present pharmacokinetic (PK) and safety results of a single ascending dose (SAD) and multiple ascending dose (MAD) study of EDP-235 in healthy subjects (HS). A phase 1, randomized, double-blind, placebo (PBO)-controlled study was conducted to assess the safety and PK profile of EDP-235 during SAD, MAD, and food effect (FE) cohorts in HS. Forty subjects were enrolled into 5 SAD cohorts (EDP-235 [n=30] or PBO [n=10], 50-800mg); thirty-two subjects were enrolled into 4 MAD cohorts dosed for 7 days (EDP-235 [n=24] or PBO [n=8], 200-800mg). EDP-235 was generally safe and well-tolerated in HS. Three discontinuations resulted from one moderate headache (400mg MAD fasted cohort), and one severe headache and one severe ALT/moderate AST elevation (800mg MAD fed cohort). EDP-235 PK increased in an approximately dose proportional manner, with T1/2 ranging from 13 to 22 hours. Exposure was enhanced ~ 4-fold with food, regardless of fat content. EDP-235 administered once daily for 7 days resulted in steady state C24 concentrations up to 6- and 13-fold the protein adjusted EC90 determined in Vero E6 cells infected with ancestral SARS-CoV-2 or the B.1.1.529 (Omicron) lineage, respectively. EDP-235 was well tolerated up to 400mg for 7 days in the fed and fasted state. Linear PK supported once daily dosing, with strong multiples over the EC90 without the need for ritonavir boosting. A Phase 2 trial of EDP-235 is planned for 4Q2022.

525. Enhanced Neutralization Escape and Fusogenicity of SARS-CoV-2 Omicron Subvariants

Shan-Lu Liu, M.D., Ph.D., *The Ohio State University, Columbus, Ohio, United States*

The development of mRNA vaccines has significantly prevented death and hospitalization during the COVID-19 pandemic, yet the continuing emergence of the SARS-CoV-2 variants has generated serious concern about the further escape from vaccine- and infection-induced immunity (Qu, NEJM, 2022; Evans, Cell Host & Microbe 2022; Evans, Science Translational Medicine 2022; Tang & Zeng, Science Immunology). In particular, newly emerged Omicron subvariants BQ.1, BQ1.1, BA.2.75.2 and XBB are responsible for the new surge of COVID-19 cases. Therefore, it is important to understand the cell tropism, fusogenicity, and immune resistance of these variants. Here we provide evidence for the enhanced neutralization resistance of all new subvariants, especially the BQ.1, BQ.1.1, and XBB subvariants driven by a key N460K mutation and to a lesser extent, R346T and K444T mutations, as well as the BA.2.75.2 subvariant driven largely by its F486S mutation. The BQ.1, BQ.1.1 and XBB subvariants also exhibited enhanced fusogenicity and S processing dictated by the N460K mutation. Interestingly, the BA.2.75.2 subvariant saw an enhancement by the F486S mutation and a reduction by the D1199N mutation to its fusogenicity and S processing resulting in minimal overall change. Molecular modelling revealed the mechanisms of monoclonal antibody-mediated immune evasion by R346T, K444T, F486S and D1199N mutations. Altogether, these findings shed light on the convergent evolution of newly emerging SARS-CoV-2 Omicron subvariants.

526. Evaluation of Molnupiravir and GS-441524 as Treatments for a Lethal Neurologic SARS-CoV-2 Infection in hACE2 Mice

Scott Gibson, Ph.D. Seeking, *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*
Ashley N. Sheesley, B.S., *Utah State University, Utah, United States*
Arnaud Van Wettere, D.V.M., Ph.D., *Utah Veterinary Diagnostic Laboratory, Utah State University, Logan, Utah, United States*
Venkatraman Siddharthan, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*
Bart Tarbet, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*
Brett L Hurst, Ph.D., *Utah State University, Logan, Utah, United States*

While SARS-CoV-2 has demonstrated the ability to infect wild-type hamsters, lethality in small animal models appears to be linked to the development of a neurologic infection and the resulting pathogenesis in the central nervous system (CNS). Several antivirals are currently available for the treatment of a SARS-COV-2 infections in humans. However, research evaluating the efficacy of these antivirals against the neurological progression of SARS-CoV-2 in humans and small animal models is lacking. Two such therapeutics are the nucleoside analogs GS-441524, the active metabolite of Remdesivir, and Molnupiravir. Transgenic hACE2 animal models such as K18-hACE2 mice are well known for the virus replication and pathogenesis observed in the CNS following SARS-CoV-2 challenge. Treatment with Molnupiravir in the lethal hACE2 mouse infection model results in a significant increase in survival over placebo-treated mice (80% vs 0%). On the other hand, GS-441524 treatment did not protect mice from a lethal SARS-CoV-2 infection in hACE2 mice. A significant reduction of infectious virus was observed in lung, brain, heart, and eye tissue of Molnupiravir-treated mice. Molnupiravir treatment also resulted in altered cytokine expression in both lung and brain tissue compared to placebo-treated mice. Our results demonstrate that treatment with Molnupiravir and not GS-441524 treatment protects mice from a lethal neurologic infection with SARS-CoV-2. This data supports the hypothesis that Molnupiravir may serve as a viable prophylactic treatment option to prevent neurological incidents of SARS-CoV-2 infection in human patients.

527. Evaluation of the Nucleoside Analogs Antiviral Potential Against SARS-CoV-2

Anastasia L. Khandazhinskaya, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*
Elena S. Matyugina, Ph.D., *Engelhardt Institute of Molecular Biology, Russia*
Vasily A. Kezin, M.S., *Engelhardt Institute of Molecular Biology, Moscow, Russia*
Sergey A. Surzhikov, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*
Ivan V. Petushkov, Ph.D., *Institute of Molecular Genetics, NRC "Kurchatov Institute", Moscow, Russia*
Andrey V. Kulbachinskiy, Ph.D., *Institute of Molecular Genetics, NRC "Kurchatov Institute", Moscow, Russia*
Sergey N. Kochetkov, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*

The SARS-CoV-2 betacoronavirus pandemic has claimed more than 6.5 million lives [1]. Despite the development and use of COVID-19 vaccines, the disease remains a major global public health problem. To date, there are no generally accepted effective methods of treating COVID-19, and therefore the creation of specific drugs for the treatment of this disease remains a very urgent task, for which various approaches are used: antibody-based drugs, inhibitors of viral enzymes (RNA-dependent RNA polymerase, proteases, etc.), inhibitors of virus entry into the cell, etc. [2]. As part of the reprofiling of existing drugs, intensive research is being carried out all over the world on drugs designed to treat other viral, bacterial and parasitic infections, autoimmune, oncological and other diseases [2]. In the context of this strategy, we screened a library of analogs of nucleosides that we had previously obtained and showed antiviral, antibacterial, antiparasitic, or antiproliferative activity. Antiviral activity was determined by the ability of the test compounds to inhibit Vero V6 cell death induced by SARS-CoV-2 virus infection with the PIC35 strain. Screening revealed leading compounds capable of inhibiting the reproduction of the SARS-CoV-2 virus with EC50 values in the range of 20–50 μM [3]. It has been shown that the compounds prevent the interaction of SARS-CoV-2 RNA-dependent RNA polymerase and RNA substrate. The structures of these compounds can be used for further optimization in order to create an antiviral drug.

1.https://covid19.who.int
2.https://covid-nma.com/living_data/index.php.
3.Matyugina E.S. et al. Acta Naturae. - 2021 - V.13(4) - P 78-81.

528. High Throughput Screen to Identify Non-Nucleoside Small Molecule Inhibitors of SARS-CoV-2 RNA-dependent RNA polymerase

Tessa Cressey, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Anand Balakrishnan, Ph.D., *United States*
Joseph Panarese, Ph.D., *United States*
Jorden Kass, Ph.D., *United States*
Yat Sun Or, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Bryan Goodwin, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*
Michael Vaine, Ph.D., *Enanta Pharmaceuticals, Watertown, MA, United States*

Developing direct-acting antivirals against SARS-CoV-2, the causative agent of COVID-19, is important for preventing severe disease. Strategies for developing small molecule antivirals include targeting non-structural proteins such as the proteases 3CLpro and PLpro, nsp13, nsp14, nsp16, and the RNA-dependent RNA polymerase (RdRp). The RdRp is

responsible for RNA replication and is an attractive target for antivirals, however nucleoside analog inhibitors may be susceptible to excision by exonuclease proofreading activity. Here, we describe a high throughput screen to identify non-nucleoside inhibitors of the SARS-CoV-2 RdRp. A biochemical assay was developed where purified recombinant RdRp consisting of nsp12, nsp7, and nsp8 elongates a primer in an annealed primer/template pair and incorporates a fluorescent UTP analog, releasing the fluorophore. Using this assay, a library of ~400,000 small molecules was screened for RdRp inhibition. After counter-screens to remove RNA intercalators, redox cyclers, and compounds with undesirable medicinal chemistry properties, 15 compounds from 11 structural families with half-maximal inhibitory concentrations (IC50s) <10 μM were selected for mechanism of inhibition studies. None of the compounds were RNA or NTP competitive inhibitors. Biochemical assays and analytical size-exclusion chromatography showed 11 compounds disrupted nsp12-nsp8 protein-protein interactions. Nano differential scanning fluorimetry analysis suggested 5 compounds target nsp12 directly. This high throughput screen identified multiple, structurally diverse, non-nucleoside SARS-CoV-2 RdRp inhibitors as potential starting points for hit optimization.

529. Identification and Preclinical Development of Kinetin as a Safe Error-prone SARS-CoV-2 Antiviral Able to attenuate Virus-induced Inflammation

Thiago Souza, Ph.D., *Fiocruz, Rio de Janeiro, Brazil*
Vagner Pinho, *Microbiológica Química e Farmacêutica, Brazil*
Carolina Queiroz Sacramento, Ph.D., *Laboratory of Immunopharmacology, Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (Fiocruz) / Center for Technological Development in Health (CDTS-Fiocruz), Rio de Janeiro, Brazil*
Rodrigo Marcon, *Centro de Inovação e Ensaios Pré-clínicos, Brazil*
Melina Heller, *Centro de Inovação e Ensaios Pré-clínicos, Brazil*
Otavio Augusto Chaves, *Fiocruz, Brazil*
Anderson Aguillón, *Microbiológica Química e Farmacêutica, Brazil*
Patrícia Momo, *Microbiológica Química e Farmacêutica, Brazil*
João Calixto, *Centro de Inovação e Ensaios Pré-clínicos, Brazil*
Jaime Rabi, *Microbiológica Química e Farmacêutica, Brazil*

Orally available antivirals against SARS-CoV-2 are still necessary because of the continuous circulation and public health impact of new variants that challenge immunized individuals. Therefore, beyond the positive antiviral clinical results with molnupiravir and Paxlovid, the continuous search for novel molecules is necessary. Since severe COVID-19 is a virus-triggered immune and inflammatory dysfunction, molecules endowed with both antiviral and anti-inflammatory activity are highly desirable. We identified here that N6-furfurylaminopurine (kinetin, MB-905) inhibits replication of SARS-CoV-2 at the submicromolar range in human hepatic and pulmonary cell lines. On infected monocytes, MB-905 reduced viral replication and IL-6 and TNFα levels. As a prodrug, MB-905 is converted into its triphosphate nucleotide to inhibit viral RNA synthesis and induce error-prone replication. Consistently, coinhibition of SARS-CoV-2 exonuclease, a proofreading enzyme, potentiated the inhibitory effect of MB-905. Oral treatment with MB-905 decreased viral replication, tissue damage and inflammation in SARS-CoV-2-infected hamsters and transgenic ACE2 mice. MB-905 showed good oral absorption, its metabolites were stable and overachieved concentrations required for in vitro inhibition in plasma and lungs. MB-905 was not mutagenic or cardiotoxic during acute or chronic treatment. Because kinetin has already been in phase 1 trial against Familial Dysautonomia at doses higher than the predicted concentrations of antiviral/anti-inflammatory activity shown here, our study suggests the opportunity for the rapid clinical development of a new orally available antiviral for the treatment of COVID-19.

530. Identifying the In Cellulo Activity of Broad-spectrum Antivirals Against Serpentoviruses

Justin G. Julander, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Camille Holt, B.S., *Institute for Antiviral Research, Utah State University, UT, United States*
Spencer Havens, B.S. Seeking, *Institute for Antiviral Research, Utah State University, Logan, UT, United States*
Tracey Logan, B.S., *Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainsville, FL, United States*
Steven Tillis, Ph.D. Seeking, *Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, United States*
Robert J. Ossiboff, D.V.M., Ph.D., *Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, United States*

Reptilian serpentoviruses are a recently discovered group that cause severe and often fatal respiratory disease in reptiles. Outbreaks of disease associated with serpentoviruses in wild lizard and turtle populations have been reported, sometimes associated with large-scale mortalities. In captivity, serpentoviruses may spread rapidly through a reptile collection and may be fatal. Unfortunately, little is known about these viruses, despite increased efforts to understand serpentovirus infection of reptiles and there are no effective treatments for infected animals. The purpose of this study was to determine the efficacy of broadly active antivirals in reducing viral load in a relevant cell line. A cell line (DPHT) derived from diamond python (Morelia s. spilota) heart cells was established that supported the growth of various serpentoviruses, including Antaresia python (APNV), ball python (BPNV), and Morelia viridis (MVNV) serpentoviruses (nidoviruses). Infection of DPHT cells resulted in measurable cytopathic effect (CPE) in 3 days after challenge. A CPE reduction assay was used to evaluate the effect of ribavirin, remdesivir, favipiravir, NITD-008, enviroximine, pirodavir and infergen against APNV, BPNV and MVNV. Remdesivir (RDV), ribavirin (RIBA) and NITD-008 (NITD) were found to have potent antiviral activity against all three serpentoviruses with SI50s ranging from 39 to 1,100 depending on compound and virus. Activity was confirmed using a virus yield reduction (VYR) assay. The other compounds showed low or no activity. These results support the further evaluation of these compounds in relevant animal models.

531. In vitro and in vivo Characterisation of Respiratory Syncytial Virus (RSV) Inhibitors Using the Example of Presatovir

Pia Thommes, *Evotec UK, Alderley Park, United Kingdom*
Haniah Abdullah, *Evotec UK, United Kingdom*
Poppy Cliffe, *Evotec UK, Alderley Park, United Kingdom*
Emma Crank, *Evotec UK, Alderley Park, United Kingdom*
Tara Langley, *Evotec UK, Alderley Park, United Kingdom*
Yvonne Newall, *Evotec UK, Alderley Park, United Kingdom*
Amy Robinson, *Evotec UK, Alderley Park, United Kingdom*
Andrew Sharp, *Evotec UK, Alderley Park, United Kingdom*
Jennifer Williams, *Evotec UK, Alderley Park, United Kingdom*

RSV infection causes serious respiratory diseases in vulnerable patients and up to 200,000 annual deaths in children under the age of five. Presatovir, a novel small-molecule was developed as treatment for RSV infection. It targets the RSV fusion protein and blocks entry by inhibiting fusion of the viral envelope with the host cell membrane. To aid the discovery of novel antiviral compounds we have developed a panel of in vitro assays and in vivo models and demonstrated their utility using presatovir. The in vitro assays with highest throughput utilised well characterised cell lines and virus isolates. For screening purposes, a luminescence assay was deployed. For further demonstration of direct antiviral effect, we have developed a cellular ELISA against different viral proteins. In addition, a classic plaque assay, as well as a higher throughput plaque staining assay demonstrated direct antiviral effect of inhibitors on number and size of viral plaques. As a more refined test system in the progression to in vivo studies we have established an RSV infection model of Mucilair™, human nasal or bronchial epithelia cultured at the air liquid interface. Treatment with presatovir demonstrated reduction of viral progeny generation as determined by RT-qPCR as well as plaque staining assay. Finally, the efficacy of presatovir was demonstrated by its effect on viral load in lungs of RSV-infected mice and cotton rats. Viral load was determined by RT-qPCR as well as plaque staining assay. Our studies confirmed the usefulness of a range of profiling assays for progression of anti-RSV inhibitors as well as the utility of presatovir as a comparator for in vitro and in vivo models.

532. In Vitro Antiviral Profile of AB-343, a Novel, Oral, Potent SARS-CoV-2 Mpro Inhibitor with Pan-coronavirus Activity

Nagraj Mani, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Dimitar Gotchev, Ph.D., *Arbutus Biopharma Inc., PA, United States*
Kayleigh R. McGovern-Gooch, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Andrzej Ardzinski, *Arbutus Biopharma Inc., Warminster, PA, United States*
Andrea Cuconati, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Rose Kowalski, *Arbutus Biopharma Inc., Warminster, PA, United States*
Muhammad Sheraz, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Holly M. Micolochick Steuer, *Arbutus Biopharma Inc., Warminster, PA, United States*
Breanna Tercero, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*

Xiaohe Wang, *Arbutus Biopharma Inc., Warminster, PA, United States*
Adam Wasserman, *Arbutus Biopharma Inc., Warminster, PA, United States*
Klaus Maskos, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
Chia-yi Chen, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
Konstanze von König, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
Andreas Schmitt, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
Archna Prasad, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
Michael Blaesse, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
DeboraL Konz Makino, *PROTEROS Biostructures GmbH, Planegg-Martinsried, Germany*
Kristi Yi Fan, *Arbutus Biopharma Inc., Warminster, PA, United States*
Steven G. Kultgen, *Arbutus Biopharma Inc., Warminster, PA, United States*
Aaron Lindstrom, *Arbutus Biopharma Inc., Warminster, PA, United States*
Duyan Nguyen, *Arbutus Biopharma Inc., Warminster, PA, United States*
Marvin Vega, *Arbutus Biopharma Inc., Warminster, PA, United States*
Xu Wang, Ph.D. , *Arbutus Biopharma Inc., Warminster, PA, United States*
Amanda Pohl, *Arbutus Biopharma Inc., Warminster, PA, United States*
Steven Ring, *Arbutus Biopharma Inc., Warminster, PA, United States*
Fran Xu, *Arbutus Biopharma Inc., Warminster, PA, United States*
Joseph Lucak, *Arbutus Biopharma Inc., Warminster, PA, United States*
Troy O. Harasym, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Andrew G. Cole, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Angela M. Lam, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*
Michael J. Sofia, Ph.D., *Arbutus Biopharma Inc., Warminster, PA, United States*

There is an urgent need for oral antiviral therapies for COVID-19. AB-343, a novel oral SARS-CoV-2 Mpro (CoV-2 Mpro) inhibitor (PI), is a potent inhibitor of its enzymatic activity (Ki 3 nM) and SARS-CoV-2 replication in vitro (EC50 ~20 nM). X-ray structural analysis of AB-343 bound to CoV-2 Mpro confirmed covalent association with the catalytic cysteine sidechain in addition to a network of interactions conferring prolonged enzyme:ligand residence time. AB-343 also demonstrates pan-coronavirus inhibition (Ki 5—45 nM against Mpro from multiple coronaviruses). Furthermore, AB-343 maintained potency against naturally prevalent CoV-2 Mpro enzyme variants including P132H (Omicron). Among variants known to confer in vitro resistance to other PIs, the Ki increases for AB-343 against CoV-2 Mpro Y54A, F140A, S144A, L167F and H172Y were modest (2–5-fold) in comparison to nirmatrelvir (25—130-fold) and ensitrelvir (15—181-fold). The E166A/V variant showed in vitro cross resistance against nirmatrelvir, ensitrelvir and AB-343 (>40-fold Ki increase). However, these variants were rare in the GISAID database and high-level in vitro resistance inversely correlated with the catalytic efficiency of CoV-2 Mpro. Moreover, AB-343 showed little to no inhibition against a human protease panel including HIV-1 protease and CoV-2 PLpro (IC50 >30 μM). AB-343 also showed no significant replication inhibition of viruses from different families with EC50/CC50 of >30 μM, except HCV (EC50 2 μM). Based on its antiviral potency, selectivity, and favorable PK, AB-343 was selected for further development as a potential ritonavir-free oral treatment for COVID-19 and other coronaviruses.

533. In vitro Characterization and Optimization of Antiviral Aerosol Therapy Against SARS-CoV-2 and Other Respiratory Viruses

Christin Müller, Ph.D., *Justus Liebig University, Institute of Medical Virology, Giessen, Germany*
Arnold Grünweller, *Philipps-Universität Marburg, Germany*
Stephan Pleschka, *Germany*
John Ziebuhr, M.D., Ph.D., *Germany*

Antiviral drug delivery by aerosol inhalation can provide several advantages over conventional therapies in combating respiratory viral infections. This includes (i) the avoidance of the liver-specific first-pass effect, (ii) a rapid onset of high local drug concentrations in the target tissue of respiratory pathogens, and (iii) minimal systemic side effects. Although aerosol inhalation is an attractive route, several pharmacokinetic and pharmacodynamic parameters need to be considered. This includes drug formulation, stability, deposition, absorption, cytotoxicity, and antiviral efficacy. In line with the 3R principle, this study aims to optimize these parameters using two previously described antiviral approaches in an ex vivo model for aerosolized drug delivery against respiratory infections. In detail, we mimicked the

human respiratory tract by using different (primary and permanent) airway epithelial cell culture models grown at an air-liquid interface, which are susceptible to viral infections. These cell models were combined with the vibrating-mesh nebulizer system ALICE-CLOUD (Air–Liquid Interface Cell Exposure–Cloud; Vitrocell®), allowing efficient in vitro aerosol application. The obtained data will help to understand whether and how nebulized antiviral compounds impair viral replication (especially SARS-CoV-2) in the airways for further development of inhalation therapy.

534. In vitro Combinations of Ribavirin with Remdesivir or GS-441524 Result In Synergistic Inhibition of hPIV3 Replication

Yuxia Lin, *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Dirk Jochmans, Ph.D., *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*

The human parainfluenza virus type 3 (hPIV3) is the most pathogenic hPIV, and only second to respiratory syncytial virus as the main cause of serious respiratory tract diseases in children, infants, and people with weakened immunity. hPIV3 causes acute low respiratory illnesses, like bronchitis and bronchiolitis, throughout the world. To date, no clinical therapies are approved for treatment of hPIV3 infection. Here, we first determined that, in LLC-MK2 cells, ribavirin, remdesivir and GS-441524 (parent nucleoside of remdesivir) inhibit hPIV3 replication in a dose-dependent manner, with EC50s of 29 μM, 0.21 μM and 1.4 μM, respectively. Next, we tested different concentration combinations of ribavirin with remdesivir or GS-441524. The combination of 13 μM ribavirin with 0.13 μM remdesivir results in 100% inhibition of hPIV3 replication, whereas the monotherapies only show 19% and 34% inhibition, respectively. This is a clear indication of synergy as the Bliss model would predict, based on these monotherapies, an inhibition of 47%. The combination of 5.0 μM ribavirin with 0.37 μM GS-441524 achieves 93% inhibition of hPIV3 replication, while the monotherapies show 16% inhibition and no significant inhibition, respectively. Taken together, this study demonstrates that the in vitro combinations of ribavirin with remdesivir or GS-441524 result in a more pronounced inhibition of hPIV3 replication than expected from the monotherapies, suggesting potential therapies for hPIV3 infection. Exploration of the antiviral potency of the combinations in human lung organoids and animal models is ongoing.

535. In Vitro Selection of SARS-CoV-2 Variants With Remdesivir or Molnupiravir Reduced Susceptibility

Carlota Fernandez-Antunez, M.S., *CO-HEP, Copenhagen, Denmark*
Kuan Wang, *Denmark*
Long Pham, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Lotte S. Mikkelsen, *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Line Abildgaard Ryberg, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Ulrik Fahnøe, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Jens Bukh, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Santseharay Ramirez, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Remdesivir and molnupiravir are broad-spectrum antivirals with high potency against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Thus, studies of their barrier to resistance are highly relevant. Here we characterized remdesivir and molnupiravir long-term exposed SARS-CoV-2 viruses in cell culture (Vero E6 cells). Compared to the original, the remdesivir exposed virus showed a 5-fold decreased remdesivir effective concentration 50% (EC50) in concentration-response assays and significantly higher infectivity titers after a 5-day treatment. It exhibited increased viral fitness in growth kinetics assays and next-generation sequencing analysis showed multiple substitutions in the genome, including nsp12-E796D (also found in a patient treated with remdesivir) and nsp14-A225S. Reverse-genetic replication experiments showed that E796D reduces remdesivir susceptibility by 3-fold (4-fold when combined with A225S) and leads to increased replication. Compared to the original, the molnupiravir exposed virus showed a

hyper-mutated viral population with a 113-, 42-, 78- and 39-fold increase in AαG, GαA, CαU and UαC transitions, respectively, and numerous substitutions across the genome. The virus maintained fitness and the population structure persisted after 10 drug-free serial passages in Vero E6 and A549-Ace2 expressing cells. It also showed increased molnupiravir EC50 (2-fold) and significantly higher infectivity titers after molnupiravir retreatment. In conclusion, SARS-CoV-2 can develop resistance to remdesivir or molnupiravir in culture, which advocates for surveillance of the emergence of escape variants after single drug therapies.

536. Localization of RNA-dependent RNA Polymerase From Picornaviruses In Cells Using Fluorescent NTPs and Synthetic Transporters

Barbora Landova, *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Vladimira Horova, *Institute of organic chemistry and biochemistry of the Czech Academy of Science, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Tomas Kraus, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Science, Prague, Czech Republic*

Positive-sense single-stranded RNA viruses are a large family that includes many dangerous human pathogens such as Yellow fever or poliovirus. The key enzyme for viral replication is the RNA-dependent RNA polymerase (RdRp). The structure of these enzymes is rather well understood. However, in infected cells RdRp always synthesizes new RNA strands on the surface of membranes that form the so called viral replication organelles [1]. Fluorescent nucleotide analogs could be used to visualize the synthesis of the new RNA strand. In this study we investigate several fluorescent nucleotide analogs whether they are incorporated by polioviral or coxackieviral RdRp into a nascent RNA strand. We choose the most promising of them and using synthetic nucleoside triphosphate transporters we deliver these NTPs into cells [2]. Using confocal microscope we observe colocalization of fluorescently labelled NTPs with fluorescently labelled replicons in cells. After replication and fixation. We mark incurred RNA nascent strands with dsRNA antibody.

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537. Low Risk of Drug-Drug Interactions (DDIs) for Bemnifosbuvir (BEM) Based Upon In Vitro Metabolism and Transporter Interaction Studies

Alex Vo, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Steven Good, Ph.D., *Atea Pharmaceuticals, Inc., Massachusetts, United States*
Jean-Pierre Sommadossi, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*
Nancy Agrawal, Ph.D., *Atea Pharmaceuticals, Inc., Boston, Massachusetts, United States*

BEM, a prodrug of a guanosine nucleotide analog, is a potent inhibitor of SARS-CoV-2 under development for treatment of COVID-19. The activation of BEM to its active triphosphate involves CatA/CES1, HINT1, ADALP1, and the kinase GUK1 and NDPK enzymes. The DDI potential of BEM and its predominant metabolites was evaluated in vitro. BEM directly inhibited Cytochrome P450 (CYP) 2C8, CYP2C9, CYP3A4m/t, and UGT1A1 with IC50 values of 51, 63, 19/83, and 44 μM, respectively. BEM was a time-dependent inhibitor of CYP3A4 with kinact of 0.0135 min-1 and Ki of 4.551 μM using midazolam as a probe substrate; and kinact of 0.0165 min-1 and Ki of 6.88 μM using testosterone as a probe substrate. None of the metabolites were direct or time-dependent inhibitors of CYP enzymes. BEM did not induce mRNA expression of CYP1A2 or CYP2B6 but was an inducer of CYP3A4 in a concentration-dependent manner. BEM was a substrate of Pgp and maybe a substrate of BCRP. BEM was not an inhibitor of OCT2 but was an inhibitor of Pgp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, MATE1, and MATE2-K with IC50 values of 14.2, 183, 33.4, 54.6, 101, 34.6, 39.7, and 195 μM, respectively. None of BEM's metabolites inhibited any transporter. Based on in vitro evaluations, there is low risk of clinically relevant DDIs when BEM is co-administered with other drugs. The

enzymes involved in the metabolic activation of BEM are of high capacity and not likely to be inhibited by commonly administered drugs. Since BEM metabolic activation does not involve CYPs, there is low risk of drug interactions as a victim of CYP enzymes. The predicted low risk of CYP- and transporter-mediated DDIs with BEM has been confirmed in clinical studies.

538. Mechanism of Action and Drug-resistance of Remdesivir, A Viral RNA Chemical Corruptor

Bhawna Sama, Ph.D. Seeking, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*
Barbara Selisko, Ph.D., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Veronique Fattorini, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Camille Falcou, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*
Géraldine Piorkowski, *Unité des Virus émergents, IRD-INSERM and Aix-Marseille University, Marseille, France*
Franck Touret, Ph.D., *UVE : Aix-Marseille University -IRD 190-Inserm 1207-IHU Méditerranée Infection, Marseille, France*
Bruno Coutard, Ph.D., *UVE : Aix-Marseille University -IRD 190-Inserm 1207-IHU Méditerranée Infection, Marseille, France*
Kim Donckers, *KU Leuven - Rega Institute, Leuven, Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Dirk Jochmans, Ph.D., *KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Lea Lo Bello, B.S., *AFMB, CNRS, Aix-Marseille University, UMR 7257, Marseille, France*
Sergio Hernandez, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*
Adrien Delpal, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*
Ashleigh Shannon, *Architecture et Fonction des Macromolécules Biologiques, CNRS and Aix Marseille Université, UMR 7257; Polytech Case 925, Marseille, France*
Bruno Canard, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*

Remdesivir (RDV) is the first FDA approved antiviral treatment for COVID-19. RDV is a nucleotide analogue carrying a ribose 1'-cyano (-CN) group and a pseudo-adenine. Nevertheless, its mode of action (MoA) against SARS-CoV-2 is still unclear. Here, we present a mechanistic analysis with SARS-CoV-2 purified enzymes; active RNA dependent RNA polymerase (RdRp) : nsp12, nsp8, nsp7 and active exonuclease (ExoN) : nsp14, nsp10 on the incorporation and excision of analogues dissecting independent contributions of RDV chemical modifications: RDV-triphosphate [RDV-TP], RDV-TP without 1'-CN [RDV(-CN)-TP], RDV where pseudo-adenine base has been replaced with an adenine base [ATP+CN]. Our biochemical assays show that the 1'-CN group allows and promotes mismatch formations suggesting mutagenic behavior. However, infected cells treated with RDV followed by NGS reveals limited direct/indirect mutagenesis. Once incorporated into RNA, RDV-MP is excised seemingly well as other RDV analogues or a natural mismatch. The 1'-CN doesn't provide any significant protection against excision. Two RDV resistance mutations selected in infected cells (S759A & A777V) yield RdRp exhibiting modest RDV discrimination but more importantly, an increased stalling during synthesis translating into more time for excision-repair. We conclude that the chemical groups of RDV incorporated into RNA have no direct effect on excision, indirectly supported by the fact that no ExoN resistance mutation has been reported so far. RDV being neither a mutagen nor chain-terminator nor excision resistant, its main MoA is the synthesis of a chemically corrupted viral RNA disturbing yet-unknown functions in the virus life cycle.

539. Mechanistic Characterization of Two Distinct Inhibitors of Coronavirus Nsp15 Endoribonuclease

Benjamin Van Loy, *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Annelies Stevaert, Ph.D., *Rega Institute for Medical Research, KU Leuven, Belgium*
Julie Vandeput, *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Gökçe Cihan-Üstündag, *University of Istanbul, Istanbul, Turkey*
TruongTien Nguyen, *Department of Chemistry, KU Leuven, Leuven, Belgium*
Gültaze Çapan, *University of Istanbul, Istanbul, Turkey*
Wim Dehaen, *Department of Chemistry, KU Leuven, Leuven, Belgium*
Kalyan Das, *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Abhimanyu Kumar Singh, *Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Arnout Voet, *Department of Chemistry, KU Leuven, Leuven, Belgium*
Lieve Naesens, Ph.D., *Rega Institute for Medical Research, KU Leuven, University of Leuven, Leuven, Belgium*

We here report on two structurally unrelated small molecules with anti-coronavirus (CoV) activity, designated ZA5 and 5h and acting via nonstructural protein 15 (nsp15). They were discovered in a screening program with HCoV-229E. In human cells infected with this virus, both molecules reduce the viral load by 10,000-fold. ZA5 is broadly active against SARS-CoV-2, HCoV-NL63 and -OC43, TGEV and MHV-A59. In contrast, the activity of 5h is restricted to HCoV-229E. Both compounds prevent the formation of dsRNA intermediates of CoV RNA synthesis. Resistant HCoV-229E mutants, obtained by serial passaging under compound, were found to carry substitutions in nsp15, specifically V42A and F230L for ZA5 and K60R and T66I for 5h. A strain of HCoV-229E bearing a catalytic site mutation in the endoribonuclease (EndoU) part of nsp15 proved markedly less sensitive to ZA5 and 5h. The two molecules showed additive effect when combined, suggesting that they do not compete for the same binding pocket within nsp15. The docking model that we constructed for 5h concurs with its resistance profile and indicates binding at an inter-monomer interface of hexameric nsp15, creating an opportunity for structure-based design of improved inhibitors. We successfully established a cryo-EM platform for nsp15, which will be instrumental to reveal the precise binding mode of ZA5 and 5h. To further decipher the mechanism of action, we are investigating whether these molecules interfere with the nsp15 EndoU function, which has a role in viral RNA synthesis and immune evasion. Nsp15 inhibition may thus have a dual pharmacological effect, making these lead compounds highly relevant for antiviral drug development.

540. Modulation of in vitro SARS-CoV-2 Infection by Stephania Tetrandra and Its Alkaloid Constituents

Stephen Polyak, Ph.D., *University of Washington, Seattle, WA, United States*
Aswad Khadilkar, *University of California at Santa Cruz, United States*
Roger Linington, *Simon Fraser University, Canada*
John MacMillan, *University of California at Santa Cruz, United States*
Nadja Cech, *University of North Carolina at Greensboro, United States*

Botanical natural products have been widely consumed for perceived promotion of health against COVID-19. Here, we screened 60 replicate preparations of six botanical extracts from multiple sources and 173 pure botanical compounds for blockade of wild type SARS-CoV-2 infection in human embryonic kidney (HEK) 293T cells over-expressing ACE-2 and TMPRSS2 protease (293TAT). Antiviral activity was demonstrated by extracts from Stephania tetrandra. Multivariate statistical analyses of fraction metabolomics data and NPanalyst software predicted that alkaloids were responsible for antiviral activity. Indeed, the alkaloid fraction and purified alkaloids tetrandrine, fangchinoline, and cepharanthine inhibited wild type SARS-CoV-2 infection in 293TAT cells. The alkaloids and alkaloid fraction also inhibited the delta variant of concern but not wild type SARS-CoV-2 in Vero E6 cells expressing TMPRSS2 and ACE-2. Membrane permeability assays suggested that the alkaloids are biologically available. At concentrations above 1 μM or 1 μg/ml, S. tetrandra extract, alkaloid fractions, and pure alkaloids induced phospholipidosis in 293TAT cells and less so in Vero E6 cells. Gene expression profiling during SARS-CoV-2 infection of 293TAT cells suggested that alkaloids could partially reverse virus-induced gene expression and pathway perturbations, with fangchinoline showing more bioactivity against virus-induced cellular perturbations than tetrandrine. Our study highlights a multi-faceted approach to systematically identify the diverse bioactivities conferred by complex botanical mixtures, their cell-context specificity, and pleiotropic effects on biological systems.

541. Molecular Scaffolds for Macro Domain Targeted Inhibition

Oney Ortega Granda, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France*
Karine Alvarez, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France*
Mikael Feracci, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
Bruno Canard, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
François Ferron, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France*
Nadia Rabah, Ph.D., *Aix Marseille Université, CNRS, AFMB UMR 7257, Marseille, France*

Coronavirus disease 2019 (COVID-2019) pandemic is an ongoing global health and economic crisis caused by human infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The SARS-CoV-2 and SARS-CoV genomes encode for 16 non-structural proteins (nsps). They drive virus replication and participate in viral evasion from the host immune response. Among coronavirus nsps, nsp3 contains a protein module termed Macro domain, which was shown to carry an IFN antagonist activity thereby interfering with host innate immunity response. This domain is able to bind and hydrolyse ADP-ribose derivatives. The hydrolysis activity is correlated to immune escape. Macro domains are involved in the regulation of a variety of physiological processes and represent valuable therapeutic targets. Based on the available structural data of the SARS-CoV-2 Macro domain, a selected set of small molecules were subjected to molecular docking in the ADP-ribose pocket. In order to screen the selected molecules an immune-enzymatic assay was developed based on the inhibition of recombinant Macro domain-ADP-ribose complex formation of SARS-CoV and SARS-CoV-2. Analogues displaying the best results in terms of ADP-ribose-binding inhibition were selected. Characterization of these molecules in the ADP-ribose pocket reveal the potential interaction with residues involved in the coordination of ADP-ribose molecule. Therefore, the results suggest the possible use of these molecules as a scaffold for the design of Macro domain specific inhibitors.

542. Morbidity and Mortality of SARS-CoV-2 Infections in PLWH in Uganda A Retrospective Cohort Study

Waiswa Moses, M.S., *Amani Children's Clinic, Kampala, Uganda*
Hakim Mugabi, M.D., *Iganga Regional Referral Hospital, Uganda*
Betty Namuland, B.Pharm, *Makerere University School of Public Health, Kampala, Uganda*

Background: Case studies suggested that no excessive morbidity or mortality of SARS-CoV-2 infections in PLWH with absence of severe immune deficiency.

Methods: Ongoing, retrospective analysis of SARS-CoV-2 infections of PLWH in Uganda, since March 2020 a non-ymized data on age, antiretroviral therapy (ART), CD4- cell count and HIV-RNA before SARS-CoV-2 infection, comorbidities', symptoms, and outcome have been collected.

Results: Until December 2020, 102 patients (84 men, 18 women, median age 47 years) were included. The median CD4 cell count was 666µl and HIV-RNA was below 50 copies/ml in 95 patients. All patients but one were on ART at SARS-CoV-2 diagnosis. Regimens contained an INSTI in 75, a PI in 18 and NNRTI in 18 cases. At least one comorbidity was reported for 66 patients, the most common were hypertension (n = 26) and diabetes (n = 10). The most frequent symptoms were cough (63%), fever (53%), and disturbance of smell or taste (28%). Of 21 hospitalized patients, 9 (43%) required intensive care. Compared to non-hospitalized patients were older (54 versus 45 years) and had lower current or nadir CD4 cell counts (490/µl versus 724/µl and 194/µl versus 374/µl, respectively).

Conclusion: In this large cohort of PLWH diagnosed with SAR-CoV-2 infection, morbidity and mortality appear to be relatively high, hospitalized patients were older and had evidence for a more pro-nounced immune deficiency (p = 00.3 for CD4 <350/µl and CD4 Nadir <200/µl using fisher's exact test). However, due to the ret-rospective design, possible confounding and a reporting bias cannot be ruled out. More data are necessary to evaluate risk factors for morbidity.

543. Mung Bean Extract Inhibits Feline Coronavirus In Vitro

Ai-Ai Chou, B.S., *National Taiwan University, Taipei, Taiwan, Republic of China*
Chia-Chen Pi, M.S., *King's Ground Biotech Co., Ltd., Taiwan, Republic of China*
Hui-Wen Chen, D.V.M., Ph.D., *National Taiwan University, Taipei, Taiwan, Republic of China*

Feline infectious peritonitis (FIP) is a fatal disease in cats caused by a mutated feline coronavirus, and none of the antivirals have been authorized for veterinary treatment. Vigna radiata (L.) R. Wilczek (mung bean) reveals pharmacological effects, and our previous study found that Vigna radiata extract (VRE) inhibits the influenza A virus

through multiple mechanisms. With the lack of treatments for FIP, VRE is investigated for its antiviral ability against feline infectious peritonitis virus (FIPV). VRE is prepared from the ethanol extraction of mung bean seed coats, and HPLC analysis showed that vitexin and isovitexin are the major components. The antiviral activity of VRE against FIPV in Felis catus whole fetus cells (Fcwf-4) was examined through the cytotoxicity test, plaque reduction test, western blot analysis and RT-qPCR. The CC50 of VRE in Fcwf-4 cells is 4539 μg/ml. VRE reduced cytopathic effects in the cytotoxicity test with an IC50 of 65 μg/ml, and it significantly decreased virus titer in a dose-dependent manner. VRE also inhibited the expression of virus nucleoprotein, as shown in western blot analysis. Besides, analyzing the virus titer of the time-of-addition assay revealed that VRE blocked FIPV in the early stage of the infection. Moreover, VRE exhibited FIPV inhibition under the antibody-dependent enhancement infection model. With the combination of VRE and GS-441524, the nucleoside analog, both drugs can function under concentrations lower than those used alone. In summary, this study characterized the anti-FIPV activity of Vigna radiata extract in vitro, and VRE possesses a therapeutic potential for FIP therapies.

544. New Fleximer aza/deaza nucleoside Analogues: Anti SARS-CoV-2 Activity and Possible Targets

Elena Matyugina, Ph.D., *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia*
Anna Varizhuk, Ph.D., *Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Russia*
Andrey V. Kulbachinskiy, Ph.D., *Institute of Molecular Genetics, NRC "Kurchatov Institute", Moscow, Russia*
Katherine L. Seley-Radtke, Ph.D., *University of Maryland, Baltimore County, Baltimore, MD, United States*
Sergey N. Kochetkov, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*
Anastasia Khandazhinskaya, Ph.D., *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia*

In the last several decades modified nucleosides have provided a wealth of drug candidates to many therapeutic areas. Nucleosides, where one of the nitrogen atom in the nucleic base is replaced with CH group, are of great interest from chemical and pharmacological point of view. Combining both deaza and aza components in a heterocyclic purine moiety also has resulted in other biologically potent purine modified nucleosides. Another strategic structural modification involves installing flexibility into the nucleobase scaffold. This class of nucleosides was named "fleximers" and have shown potent activity against a number of viruses, including Ebola, Marburg, MERS-CoV, SARS-CoV2 Dengue, and Yellow Fever. Here we present a series of new nucleoside analogues which combine two structural modifications of the heterocyclic bases mentioned above. During wide antiviral screening compounds with anti SARS-CoV2 activity were revealed. IC50 for 1-(β-D-ribofuranosyl)-4-(4-aminopyrimidin-5-yl)pyrazole was 80 μM. It's triphosphate was synthesized and studied as inhibitor of RNA-depended RNA-polymerase. It was shown that when using ATP as a substrate at a concentration of 3 μm, some (not complete) inhibition occurs at high concentrations of triphosphate (300 μm). Besides fleximers were studied as modulators of intracellular liquid-liquid phase separation (LLPS) of SARS-CoV-2 nucleocapsid protein (N) and RNA which is important for the viral life cycle and considered as new druggable target. Fleximers turned out to be mildly efficient LLPS modulators, comparable to N(4)-hydroxycytidine.
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545. Nirmatrelvir Resistant SARS-CoV-2 Variants with High Fitness in an Infectious Cell Culture System

Yuyong Zhou, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Hvidovre, Denmark*
Karen Anbro Gammeltoft, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Line Abildgaard Ryberg, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Long Pham, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

Helena Tjørnelund, M.S., *Department of Chemistry, Technical University of Denmark, 2800 Kongens Lyngby, Denmark, Denmark*
Alekxander Binderup, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Carlos Duarte Hernandez, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Carlota Fernandez-Antunez, M.S., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Anna Offersgaard, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Ulrik Fahnøe, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Günther Peters, Ph.D., *Department of Chemistry, Technical University of Denmark, Kongens Lyngby, Denmark*
Santseharay Ramirez, Ph.D., *Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital–Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*
Jens Bukh, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*
Judith M. Gottwein, M.D., *Copenhagen Hepatitis C Program (CO-HEP), Copenhagen, Denmark*

The oral protease inhibitor nirmatrelvir is of key importance for prevention of severe coronavirus disease 2019 (COVID-19), however, it's efficacy might be threatened by development of resistance. To investigate the potential of resistance development of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to nirmatrelvir, we studied SARS-CoV-2 escape from nirmatrelvir in cell culture. Nirmatrelvir resistant escape variants harbored combinations of substitutions in the SARS-CoV-2 main protease (Mpro) which conferred up to 175-fold resistance. In an infectious cell culture system, as well as in replicon- and protease-assays, reverse genetics revealed that E166V and L50F+E166V conferred high resistance to nirmatrelvir. Specifically, in short-term infectious and protease antiviral treatment assays, L50F+E166V conferred up to 80- and 316-fold resistance, respectively. L50F and L50F+E166V variants maintained high fitness and were genetically stable in the infectious system, despite these mutations decreasing viral replication and Mpro activity. Naturally occurring L50F compensated for fitness cost of E166V and promoted viral escape. Molecular dynamics simulations revealed that E166V and L50F+E166V weakened nirmatrelvir-Mpro binding. Polymerase inhibitor remdesivir and monoclonal antibody bebtelovimab retained activity against nirmatrelvir resistant variants and combination with nirmatrelvir enhanced treatment efficacy compared to individual compounds. Mpro L50 and E166 are highly conserved for sarbecoviruses, thus, these findings have implications for monitoring and ensuring treatments with high efficacy against SARS-CoV-2 and against future emerging sarbecoviruses.

546. Novel Attachment Inhibitors of Human Parainfluenza 3

Gregory Mathez, M.S., *Institute of Microbiology, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland*
Jacob Silva Paulo Henrique, *EPFL, Switzerland*
Francesco Stellacci, *Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
Valeria Cagno, Ph.D., *CHUV, Lausanne, Switzerland*

Human parainfluenza is one of the major respiratory viral infections in young children. Human parainfluenza 3 (PIV3) is the most common type. Unfortunately, PIV3 infection can cause bronchitis and pneumonia leading to hospitalization. No vaccine nor antivirals are available. With the aim of finding new antivirals, we designed attachment inhibitors.

To be representative of the current PIV3 circulation, we performed a glycan array with two clinical isolates from university hospitals in Switzerland and with an ATCC strain. LS tetrasaccharide D (LSTd), a sialic acid-based glycan present in the human respiratory tract, and heparin octasacharide were the top common hits. We synthesized modified cyclodextrin harboring LSTd (CD-LSTd) or sulfonates (CD-MUS). The structure of the macromolecule was chosen from previous work in which it was shown to confer not only antiviral activity but also virucidal activity. Both molecules showed antiviral and virucidal activity, although the sulfonated had higher potency. They retained activity in human-derived respiratory tissues. Combination and structural experiments suggest different proximal binding sites. Optimization of therapeutical administration in ex vivo upper respiratory tract model is ongoing with the future goal of in vivo tests. Altogether, we designed promising new antivirals active against clinical PIV-3 with a broad-spectrum activity against other respiratory viruses.

547. Novel SARS-CoV-2 nsp14 Inhibitors as Potential Antiviral Compounds

Radim Nencka, Ph.D., *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*
Tomáš Otava, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Milan Štefek, *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Milan Dejmek, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Evzen Boura, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Dominika Chalupská, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*
Karel Chalupsky, Ph.D., *Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic*

The COVID-19 pandemic caused SARS-CoV-2 infection represents one of the biggest challenges to global health today. There are currently some drugs on the market that directly target SARS-CoV-2 viral proteins, but the mutational potential of this dangerous virus and our experience with viral infections such as HIV and HCV suggest that the most effective therapy will be based on a combination of drugs with multiple different mechanisms of action. Viral methyltransferases have been a major interest of our research group for several years and may represent one such attractive molecular target against not only SARS-CoV-2 but also other viral pathogens. Here, we will present some new types of inhibitors of one of the SARS-CoV-2 methyltransferases – 7N-methyltransferase, which is a part of the nsp14 protein. We will focus in particular on the rational design of these compounds and their biological activity.

548. Peruvian Amazonian Medicinal Plants With Antiviral Activities Against Human Coronavirus

Karin Séron, Ph.D., *Center for Infection and Immunity of Lille, Lille, France*
Lowiese Desmarets, Ph.D., *Center for Infection and Immunity of Lille (CIIL), France*
Vincent Roumy, *UMRT 1158 BioEcoAgro- Univ Lille, France*
Lastenia Ruiz Mesia, *Centro de Investigación de Recursos Naturales, Universidad Nacional de la Amazonía Peruana, Peru*
Nathan François, *Center for Infection and Immunity of Lille (CIIL), France*
Charline Camuzet, *Center for Infection and Immunity of Lille, France*
Jean Dubuisson, *Center for Infection and Immunity of Lille (CIIL), France*
Sandrine Belouzard, *Center for Infection and Immunity of Lille (CIIL), France*

According to WHO, more than 80 % of the world population relies on traditional medicine for primary health care. Peruvian Amazonian territory is still inhabited by remote indigenous populations, who are using herbal traditional medicines. Despite their isolation, they were dramatically affected by the COVID-19 pandemic. The aim of our study was to identify plants used in traditional medicine with antiviral activities against human coronaviruses. Ethnobotanical surveys were conducted to collect plant species used in contemporary phytotherapies by native and neo-urban societies from Iquitos surroundings against infectious diseases. 151 crude plant extracts were obtained and antiviral screening was performed with a luciferase recombinant HCoV-229E in Huh-7 cells. Plant extracts significantly reducing HCoV-229E infection were selected and tested against SARS-CoV-2 in Vero-81 cells by quantification of the SARS-CoV-2 N protein expression levels in the treated samples. Extract cellular toxicity was monitored in both cell lines by MTS assays. Among the 151 crude extracts tested, 36 of them significantly reduced HCoV-229E infection in Huh-7 cells at 25 µg/mL. Of these extracts, 11 were able to reduce SARS-CoV-2 infection at 25 µg/mL. No toxicity was observed at this concentration. Experiments are underway to determine precise IC50 and CC50 values of each extract for both HCoV-229E and SARS-CoV-2 in order to identify the most promising extracts. In conclusion, some very interesting pancoronavirus antiviral activities have been identified in plant extracts from Peruvian Amazonian traditional herbal medicine used against infectious diseases.

549. Pharmacokinetics and Metabolism of [14C]-bemnifosbuvir In Healthy Male Participants

Xiao-Jian Zhou, Ph.D., *Atea Pharmaceuticals, Inc., Boston, MA, United States*
Maureen Montrond, B.S., *Atea Pharmaceuticals, Inc., MA, United States*
Keith Pietropaolo, B.S., *Atea Pharmaceuticals, Inc., Boston, MA, United States*
Bruce Belanger, Ph.D., *Atea Pharmaceuticals, Inc, Boston, MA, United States*
Arantxa Horga, M.D., *Atea Pharmaceuticals, Inc., Boston, MA, United States*
Janet Hammond, M.D., Ph.D., *Atea Pharmaceuticals, Inc., Boston, MA, United States*

Bemnifosbuvir (BEM, AT-527) is a guanosine nucleotide prodrug candidate for the treatment of COVID-19 and chronic HCV. Results from a human mass balance study characterizing the absorption, metabolism and elimination profiles of BEM in healthy male participants are reported. Six healthy male participants were enrolled and received a single oral dose of BEM 550mg containing 100 µCi [14C]-BEM. Serial whole blood, plasma, urine and fecal samples were collected for up to 216h post dose and quantitated by radioactivity counting and/or using LC/MS/MS methodologies. Recovery of radioactivity was near complete with 64.1% and 25.2% recovered from urine and feces totaling 89.2% of dose. Fraction absorbed was estimated to be 65%. Total blood and plasma radioactivity over time curves overlapped with blood to plasma ratios (0.8-1.1), indicative of no red blood cell (RBC) accumulation. Plasma, urine and fecal samples were subject to metabolite identification, confirming formation of AT-551, the L-alanyl intermediate and two nucleoside metabolites AT-229 with 6N-modification and AT-273, the guanosine metabolite. Upon oral administration, BEM was rapidly absorbed and extensive metabolized to AT-551. AT-229 was the most abundant whereas AT-273 exhibited a sustained plasma profile with a mean half-life of 19.7h. Both nucleoside metabolites were largely eliminated in urine, with estimated renal clearance exceeding glomerular filtration rate. BEM was well absorbed with administered dose nearly completely recovered in urine and feces, with no RBC accumulation. BEM was extensively metabolized to AT-551, underwent additional metabolism entering general circulation as mostly AT-229 and AT-273

550. Potent Phosphoramidate Prodrugs of the Remdesivir Parent Nucleoside GS 441524) for Intramuscular Injection

Rao V. Kalla, Ph.D., *Gilead Sciences, Foster City, California, United States*
Scott Simonovich, Ph.D., *Gilead Sciences, Foster City, California, United States*
Thao Perry, M.S., *Gilead Sciences, Foster City, California, United States*
Kwon Chun, Ph.D., *Gilead Sciences, Foster City, California, United States*
Hon C Hui, M.S., *Gilead Sciences, Foster City, California, United States*
Dmitry Koltun, Ph.D., *Gilead Sciences, Foster City, California, United States*
Daniel Byun, M.S., *Gilead Sciences, Foster City, California, United States*
Petr Jansa, Ph.D., *Gilead Sciences, Foster City, California, United States*
Julie Chan, M.S., *Gilead Sciences, Foster City, California, United States*
Bruno Marchand, Ph.D., *Gilead Sciences, Foster City, California, United States*
Venice Du Pont, Ph.D., *Gilead Sciecnes, Foster City, California, United States*
Xianghan Lu, M.S., *Gilead Sciences, Foster City, California, United States*
Jared Pitts, Ph.D., *Gilead Sciences, Foster City, California, United States*
Xiaoffeng Zhao, M.S., *Gilead Sciences, Foster City, California, United States*
Darius Babusis, Ph.D., *Gilead Sciences, Foster City, California, United States*
Diane Lye, Ph.D., *Gilead Sciences, Foster City, California, United States*
Dustin Seigel, Ph.D., *Gilead Sciences, Foster City, California, United States*
Michael O. Clarke, Ph.D., *Gilead Sciences, Foster City, California, United States*
Raju Subramanian, Ph.D., *Gilead Sciences, Foster City, California, United States*
John P. Bilello, Ph.D., *Gilead Sciences, Foster City, California, United States*
Richard L. Mackamn, Ph.D. , *Gilead Sciences, Foster City, California, United States*

Remdesivir (RDV) is the first FDA approved agent for the treatment of COVID 19 and is administered through a 3–5-day regimen of once daily IV infusions. Once inside cells, the phosphoramidate prodrug of RDV is metabolized to the nucleoside monophosphate and subsequently to the active triphosphate metabolite (NTP) inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase. We reasoned that modification of the phosphoramidate prodrug could generate higher and more persistent NTP concentrations inside lung cells, which would enable a single dose intramuscular (IM)

product. Therefore, we embarked on an optimization campaign replacing the phenol and 2-ethyl butyl ester components of the monoalanine phosphoramidate prodrug of RDV to arrive at GS-1152808. In vitro, GS-1152808 demonstrated 2-5-fold improved potency over RDV toward RSV and SARS-CoV-2 in relevant cell types, including primary lung cells, concomitant with its higher intracellular NTP levels. Intramuscular administration in cynomolgus monkeys at an approximately equivalent dose level, however, demonstrated lower NTP concentrations in lung tissue at 24h post dose compared to RDV. One hypothesis for this result is that the higher plasma protein binding of GS-1152808 (~99.5%) potentially restricted in vivo metabolism and effective loading of lung cells compared to RDV (90% protein-bound). Further research is needed to optimize lung delivery following IM injection for the more potent amidate prodrugs.

552. SARS-CoV-2 Perturbs Polyamine Metabolism To Ensure Efficient Replication: Studies Using Plasmax Culture Medium

Alexander V. Ivanov, Ph.D., *Engelhardt Institute of Molecular Biology, Moscow, Russia*
Mikhail V. Golikov, M.S., *Engelhardt institute of Molecular Biology, Moscow, Russia*
Olga N. Ivanova, Ph.D., *Engelhardt institute of Molecular Biology, Moscow, Russia*
Olga A. Smirnova, Ph.D., *Engelhardt institute of Molecular Biology, Moscow, Russia*
Birke Bartosch, Ph.D., *Lyon Cancer Research Center, Lyon, France*
Dmitry V. Yanvarev, Ph.D., *Engelhardt institute of Molecular Biology, Moscow, Russia*

Cell metabolism is tightly linked with signaling pathways, cell growth and differentiation. Various pathologies are associated with alterations in metabolic pathways. Many viruses interfere with host cell metabolism. However, investigation of metabolism is hampered by imprint of classical culture medium. We showed that a recently developed plasma-resembling Plasmax medium supports replication of various viruses including HCV, influenza virus and SARS-CoV-2 and ensures their more pronounced effect on redox landscape of cells than classical media. Moreover, this medium significantly increased rates of respiratory activity and decreased lysosomal mass of uninfected cells. Next, we explored the effect of SARS-CoV-2 on transcriptome of A549-ACE2 and Huh7.5 cells. It revealed that the virus strongly induces expression of ornithine decarboxylase (ODC) - the rate-limiting step of polyamine biosynthesis. In classical media this effect much was profound. It led to elevation of polyamines levels in the infected cells. The inhibitors of ODC as well as of polyamine oxidases suppressed replication of SARS-CoV-2 showing that polyamines are necessary for replication of the coronavirus. These data not only reveal interference of SARS-CoV-2 with host cell metabolic pathways but also demonstrate importance of usage of plasma-like media in virology.
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553. Sars-Cov-2 Viruses with Cross-Resistance to 3CLpro Inhibitors Can Be Selected In Vitro, and Can Replicate and Transmit in a Hamster Model

Dirk Jochmans, Ph.D., *KU Leuven - Rega Institute, Leuven, Belgium*
Rana Abdelnabi, Ph.D., *Rega Institute for Medical Research, Belgium*
Kim Donckers, *KU Leuven - Rega Institute, Leuven, Belgium*
Cheng Liu, *Aligos Therapeutics, Inc., CA, United States*
Antitsa Stoycheva, *Aligos Therapeutics, Inc., CA, United States*
Sandro Boland, *CISTIM, Leuven, Belgium*
Sarah Stevens, *Aligos Therapeutics, Inc., CA, United States*
Chloe De Vita, *Aligos Therapeutics, Inc., CA, Belgium*
Bettina Trüeb, *University of Bern, Bern, Switzerland*
Nadine Ebert, *University of Bern, Bern, Switzerland*
Volker Thiel, *University of Bern, Bern, Switzerland*
Manon Laporte, Ph.D., *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Steven De Jonghe, Ph.D., *KU Leuven, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Dorothée Bardiot, *CISTIM, Leuven, Belgium*
Andreas Jekle, *Aligos Therapeutics, Inc., CA, United States*
Lawrence Blatt, *Aligos Therapeutics, Inc., CA, United States*
Leonid Beigelman, *Aligos Therapeutics, Inc., CA, United States*
Julian Symons, *Aligos Therapeutics, Inc., CA, United States*
Patrick Chaltin, *Centre for Drug Design and Discovery (CD3), KU Leuven, Bioincubator 2 and Cistim Leuven vzw, Bioincubator 2, Leuven, Belgium*
Arnaud Marchand, *CISTIM, Leuven, Belgium*

Previously we reported that ALG-161, a SARS-CoV-2 3CL-protease (3CLpro) inhibitor, can select for resistance in vitro by acquiring the L50F+E166A+L167F substitutions in the 3CLpro protein (Jochmans et al. 2022). Additional in vitro selection experiments were performed with nirmatrelvir (Paxlovid) and ensitrelvir. Nirmatrelvir selected for viruses with one or more of the following 3CLpro substitutions: T21, D48N, L50F, S121L, S144A, E166V, E166A, A173V and T304I (n=6). Ensitrelvir selected for viruses with D48G, M49I, S144A and T169I (n=3). The effect of all these substitutions and their combinations remains to be investigated, but in a first instance we engineered, using an infectious clone, the 3CLpro L50F+E166A+L167F virus and performed a full characterization. In vitro, this virus shows high resistance (>10x) against nirmatrelvir, ensitrelvir and ALG-161. In vivo, hamsters can be efficiently infected with this virus, leading to an infectious viral load of 3.2 Log10 TCID50/mg lung on day 4 p.i. (compared to 5.1 Log10 TCID50/mg for WT virus). This virus is also efficiently transmitted to co-housed sentinels with 9 out of 12 hamsters being infected at 4 dpi (compared with 11/12 for WT) (Abdelnabi et al. 2022). Sequence analysis of the transmitted virus shows that the resistance-associated mutations are preserved in vivo in the absence of compound exposure. In conclusion, we demonstrate that 3CLpro inhibitors can select for different mechanisms of (cross)-resistance in vitro. Similar results have been obtained by other groups (Yuyong et al., Sho et al.2022). We also demonstrate that 3CLpro-inhibitor resistant virus can replicate and transmit in the hamster model.

554. Structure-guided Design of Protease-resistant, Lipopeptide Inhibitors of SARS-CoV-2

Ariel Kuhn, Ph.D., *University of Wisconsin - Madison, Madison, WI, United States*
Samuel Gellman, Ph.D., *University of Wisconsin - Madison, Madison, WI, United States*
Anne Moscona, M.D., Ph.D., *Columbia University - Department of Microbiology & Immunology, New York, NY, United States*
Matteo Porotto, Ph.D., *Department of Pediatrics, Center for Host-Pathogen Interaction, Columbia University Medical Center, New York, NY, United States*

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has spurred an unprecedented global pandemic, necessitating new therapeutic agents that are effective against a rapidly changing virus. To meet this end, our team of chemists and virologists has pioneered an approach for developing peptide-like inhibitors with improved pharmacokinetic properties. SARS-CoV-2 infection is instigated by spike (S) protein-mediated fusion between the viral envelope and the host cell membrane. This mechanism is facilitated by the rearrangement of the S homotrimer into a stable, 6-helical bundle (6HB), formed between two heptad repeat domains, HRN and HRC. Peptides derived from SARS-CoV-2 S HRC can inhibit viral infection through formation of a stable, hybrid-6HB. However, conventional peptides, composed entirely of α-amino acids, are susceptible to proteolytic degradation. We have demonstrated, in collaboration with Anne Moscona and Matteo Porotto, that site-selective incorporation of backbone modifications, such as β-amino acids, in combination with cholesterol conjugation, can decrease proteolytic sensitivity while maintaining high pan-variant, antiviral potency. Resultant backbone-modified lipopeptides have been evaluated in a cell-based assay for S-mediated membrane fusion, which is strongly predictive of in vivo efficacy. Structural studies in the areas of protein crystallography, circular dichroism and fluorescence polarization, have further assisted us in making design improvements. Through structure-guided engineering and multi-pronged assay implementation, these efforts could generate effective pan-variant therapeutics for COVID-19.

555. Targeting the Host Cell Metabolism to fight Respiratory Viral Infections

Anna-Dorothea Gorki, Ph.D., *G.ST Antivirals GmbH, Vienna, Austria*
Laxmikant Wali, M.S., *G.ST Antivirals GmbH, Austria*
Vitalii Kovtunyk, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Scharon Chou, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Michael Karbiener, Ph.D., *Takeda Manufacturing Austria AG, Vienna, Austria*
Delyana Stoeva, M.S., *G.ST Antivirals GmbH, Vienna, Austria*
Nicole Doyle, Ph.D., *The Pirbright Institute, United Kingdom*
Irene Gösler, *Department of Medical Biochemistry, Max F. Perutz Laboratories, Vienna, Austria*
Dieter Blaas, Ph.D., *Department of Medical Biochemistry, Max F. Perutz Laboratories, Vienna, Austria*
Johannes Stöckl, Ph.D., *G.ST Antivirals GmbH, Vienna, Austria*
Thomas R. Kreil, Ph.D., *Takeda Manufacturing Austria AG, Vienna, Austria*
Helena Maier, Ph.D., *The Pirbright Institute, Woking, United Kingdom*
Guido A. Gualdoni, M.D., Ph.D., *G.ST Antivirals GmbH, Vienna, Austria*

Rhinoviruses and coronaviruses are the leading cause of respiratory tract infections ranging from common cold to exacerbations in chronic obstructive pulmonary disease (COPD) patients. Their rapid viral replication cycle poses challenges to antiviral drug development due to quick transmission and higher mutation rate leading to resistance development. The glucose analogue, 2-deoxy-D-glucose (2-DG), acts as a host-directed antiviral agent by inhibiting glycolysis and nutrient flow within the cell needed for viral replication. We found a dose-dependent reduction of infectious particles in 2-DG treated cells for all RV and CoV strains tested, including SARS-CoV-2. To understand which steps of the virus replicative cycle are influenced by 2-DG, we measured viral RNA strands and applied microscopy to understand the formation of viral replication organelles. Our data suggests an influence of 2-DG on early stages of viral infection, specifically on virus RNA replication. In addition, we could show a significant protection against virus-induced cell death by 2-DG. Aside from the antiviral effect, we also investigated the impact of 2-DG on pro-inflammatory responses as the drivers of symptoms in respiratory infections. We observed a downregulation of NF-kB activation in activated human monocytic THP1 cells pretreated with 2-DG. Next, we used air-liquid interface models of bronchial epithelium from healthy donors treated with cigarette smoke extract to study the effect of 2-DG in a setting mimicking viral-induced exacerbations in COPD patients. Overall, the results indicate that focusing on the host cell metabolism is a promising approach to target respiratory viral infections.

556. Targeting the Interaction Between host MASP-2 and the Viral N Protein as a Broad-spectrum Therapeutic Approach for Coronavirus Infections

Ben Flude, Ph.D. Seeking, *Swansea University, Swansea, United Kingdom*
Giulio Nannetti, Ph.D., *Swansea University, United Kingdom*
Gilda Padalino, Ph.D., *Cardiff University, United Kingdom*
Martin Jacob Necesito, M.S., *United Kingdom*
Andrea Brancale, Ph.D., *Czech Republic*
Marcella Bassetto, Ph.D., *Swansea University, United Kingdom*

Several mechanisms contribute to disease progression and outcome in Covid-19 patients, with one being mediated by the interaction between SARS-CoV-2 N protein and the host MASP-2 serine protease. The amino acid sequence in the N protein responsible for this interaction is conserved in highly pathogenic SARS-CoV-2, MERS-CoV and SARS-CoV, and in different bat coronaviruses, while it is absent in human coronaviruses associated with mild disease. The interaction triggers over-activation of the complement system, leading to aggravated lung inflammation, severe pneumonia, and lung injury. Its inhibition represents a promising therapeutic strategy to interfere with the serious health complications of coronavirus infections presently affecting humans, and of those possibly emerging in the future. A computational model for the N protein-MASP-2 interaction was built and used to screen in silico commercial compound libraries, which also included drug repurposing candidates. In addition, two ligand-based approaches were applied to identify inhibitors of such interaction: the N protein portion interacting with MASP-2 was used for a shape-based virtual screening, and to design new peptidomimetics with the key functional groups of this sequence. In parallel, an ELISA assay was developed to specifically detect the interaction between MASP-2 and SARS-CoV-2 N protein and test the inhibitory activity of the compounds identified in silico and purchased, and of the novel peptide mimics. In this presentation, the rationale behind our approach will be discussed, along with the virtual screenings performed, the synthesis of the new peptidomimetics, and the findings obtained in the ELISA assay.

557. Targeting the Main Protease to Develop the SARS-CoV-2 Antivirals

Wenshe Liu, Ph.D., *Texas A&M University, College Station, TX, United States*
Shiqing Xu, Ph.D., *Texas A&M University, College Station, TX, United States*

COVID-19 is the currently prevailing pandemic. Its pathogen is SARS-CoV-2. SARS-CoV-2 relies on its main protease (Mpro) to process multiple gene translates that are essential for the viral replication and virion packaging. Being an essential enzyme, Mpro is a perfect target to develop SARS-CoV-2 antivirals. Mpro has a catalytic cysteine that can be targeted by covalent warheads. Through the development of both reversible and irreversible inhibitors with covalent warheads including aldehyde, nitrile, azapeptidyl chloroacetamide, benzothiazolylketone, and acetoxymethylketone for MPro, we have developed a number of potential SARS-CoV-2 antivirals. Some of these inhibitors showed IC50 values below 1 nM and antiviral EC50 values below 100 nM. Through high-throughput screening of a collection of 80,000 small molecules, we have also pulled out many small molecules with novel chemical functionalities that form covalent adducts with Mpro. Halicin, a recently identified pan-antibiotics, covalently conjugates to the Mpro active site cysteine through an SNAr reaction, a mechanism that has not been explored in the development of SARS-CoV-2 antivirals.

558. Tracking SARS-CoV-2 variants for monoclonal therapeutic antibodies evaluation

Franck Touret, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Jean-Sélim Driouich, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, France*
Cécile Baronti, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Antoine Nougairède, Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*
Xavier de Lamballerie, M.D., Ph.D., *Unité des Virus Émergents, UVE: Aix Marseille Univ, IRD 190, INSERM 1207, Marseille, France*

Therapeutic antibodies were among the first counter measures developed to ease the burden of severe SARS-CoV-2 infections. They were initially developed based on the ancestral SARS-CoV-2 spike. Their activity has been altered by the emergence of various variants. The most significant decrease in the number of available therapeutic antibodies was the emergence of the first Omicron (BA.1) in which six of these antibodies have totally lost their ability to neutralize it. Of the antibodies still having neutralizing activity, Sotrovimab shows the smallest reduction in activity. Cilgavimab alone shows a reduction in efficacy with the loss of tixagevimab activity, resulting in a significant loss of activity for the Evusheld cocktail. Then, new Omicron subvariants emerged (BA.2 and BA.5) and made the use of remaining active antibodies even more complicated. Sotrovimab is less active against BA.2 than against BA.1 and even less active against BA.5. Within the Evusheld cocktail, Cilgavimab is more active against BA.2 and BA.5 than against BA.1. In total, compared to BA.1, the activity of the Evusheld is significantly improved against BA.2 and BA.5. Finally, an unexpected increase in the number of subvariants carrying similar mutations to escape humoral immunity emerged. Sotrovimab retains some activity against BA.2.75.2, BQ.1 and XBB.1 as it did against BA.2/BA.5, but is less active against BQ.1.1, which was confirmed, in vivo in the hamster model. Within the Evusheld cocktail, Cilgavimab lost all activity against all subvariants studied, resulting in a loss of Evusheld activity.

559. Treatment of EV-D68 Neurological Disease in IFNAR Mice with EIDD-1931 and Human Intravenous Immunoglobulin

Brett Hurst, Ph.D., *Utah State University, Logan, Utah, United States*
Zoe Taylor, B.S., *Utah State University, Utah, United States*
Scott A. Gibson, M.S., *Utah State University, Logan, Utah, United States*
Bart Tarbet, Ph.D., *Institute for Antiviral Research, Utah State University, Logan, Utah, United States*

Enterovirus D68 (EV-D68) emerged as a pathogen of consequence in 2014 due to an outbreak of 1,395 cases of respiratory disease and temporal association with acute flaccid myelitis (AFM) cases characterized by muscle weakness and paralysis. In the most recent outbreak in 2018, more than 90% of patients with AFM had a mild respiratory illness or fever prior to developing AFM. We evaluated four isolates of EV-D68 obtained in 2018 for neurovirulence and lethality in 4- to 7-day-old IFNAR mice which are deficient in a- and b-interferon receptors. Of the four viruses evaluated, consistent mortality was observed with the US/2018-23209 (MD) and US/2018-23216 (NY) isolates of EV-D68. Only partial mortality was observed in 4- to 7-day-old IFNAR mice infected with the US/2018-23263 (MN) and US/2018/23201 (WA) isolates of EV-D68. Due to the consistent mortality observed, the US/2018-23209 (MD) isolate was used to evaluate the efficacy of EIDD-1931 or human intravenous immunoglobulin (hIVIG) to prevent mortality and paralysis of IFNAR mice. A 100 mg/kg/d dose of EIDD-1931 protected seven of ten mice (70%) from mortality and reduced neurological scores which indicate severity of paralysis. Doses of 30 or 10 mg/kg/d of EIDD-1931 did not provide a significant survival benefit to IFNAR mice infected with EV-D68. A single 100 mg/kg dose of hIVIG completely protect mice from mortality and reduced neurological scores. These data support further research into EIDD-1931 and hIVIG to reduce neurological disease due to EV-D68 infection. [Supported by Contract HHSN272201700041I from the Virology Branch, DMID, NIAID, NIH].

560. Trypthophan Derivatives Block SARS-CoV-2 Entry by Interfering the Interaction of ACE2 with the Viral Spike

María-Jesús Pérez-Pérez, Ph.D., *Instituto de Quimica Medica (IQM, CSIC), Madrid, Spain*
Marta Gargantilla, Ph.D., *Instituto de Quimica Medica (IQM, CSIC), Spain*
Clara Francés, M.S., *Instituto de Biología Integrativa de Sistemas (I2SysBio, UV-CSIC), Valencia, Spain*
Anmol Adhav, M.S., *Instituto de Biomedicina de Valencia (IBV, CSIC) and CIBERER-ISCIII, Valencia, Spain*
Alicia Forcada-Nadal, Ph.D., *Instituto de Biomedicina de Valencia (IBV, CSIC) and CIBERER-ISCIII, Valencia, Spain*
María-Luisa López-Redondo, Ph.D., *Instituto de Biomedicina de Valencia (IBV, CSIC) and CIBERER-ISCIII, Valencia, Spain*
Olaia Martí-Marí, Ph.D., *Instituto de Química Médica, Spanish Research Council, Madrid, Spain*
Juan-Carlos Saiz, Ph.D., *Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, CSIC), Madrid, Spain*
Miguel A. Martín-Acebes, Ph.D., *Department of Biotechnology, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, CSIC), Madrid, Spain*
Alberto Marina, Ph.D., *Instituto de Biomedicina de Valencia (IBV, CSIC) and CIBERER-ISCIII, Valencia, Spain*
Vicente Rubio, M.D., Ph.D., *Instituto de Biomedicina de Valencia (IBV, CSIC) and CIBERER-ISCIII, Valencia, Spain*
José-Luis Llacer, Ph.D., *Instituto de Biomedicina de Valencia (IBV, CSIC) and CIBERER-ISCIII, Valencia, Spain*
Ron Geller, Ph.D., *Instituto de Biología Integrativa de Sistemas (I2SysBio, UV-CSIC), Valencia, Spain*
Ana San Felix, Ph.D., *Instituto de Quimica Medica (IQM, CSIC), Madrid, Spain*

Spike proteins, crucial for the entry process into the host cells, characterize coronaviruses. In the case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, the viral spike through its receptor binding domain (RBD) interacts with the human angiotensin-converting enzyme (hACE2) membrane receptor to initiate infection. Thus, blocking the spike-hACE2 interaction represents an appealing antiviral strategy that could be combined with already approved treatments. We have screened an in-house library of multivalent tryptophan derivatives as SARS-CoV-2 entry inhibitors using VSV pseudoparticles expressing the SARS-CoV-2 spike protein in Vero cells. The identified hits have been explored through the synthesis of structural analogues, compounds that have shown IC50 values in the low micromolar or submicromolar range in the absence of toxicity to host cells (CC50>100 μM). Their antiviral activity has been confirmed against SARS-CoV-2 in infected Vero E6 and A549-ACE2 cells. Thermofluor and microscale thermophoresis have proven the binding of these compounds to viral RBD and spike and their capacity to interfere with hACE2 binding. Cryo-EM structure determinations of a spike-inhibitor complex has revealed plausible structural mechanisms of drug binding and inhibition. Finally, an analogue modified to improve water solubility has been chosen for efficacy studies in a transgenic mouse model expressing the hACE2 by intranasal administration. The chemical structures of these inhibitors will be disclosed. Funded by the EC – NextGenerationEU (Regulation EU 2020/2094) CSIC's Global Health Platform.

561. Umifenovir and Interferon -alpha-2b Are Broadly Effective Against SARS-CoV-2 Variants

Irina A. Leneva, Ph.D., *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*
Artem A. Poromov, Ph.D., *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*
Nadezhda P. Kartashova, Ph.D. Seeking, *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*
Ekaterina A. Glubokova, Ph.D. Seeking, *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*
Anastasia V. Gracheva, Ph.D. Seeking, *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*
Olga V. Borisova, Ph.D., *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*
Evgeny B. Faizuloev, Ph.D., *I.Mechnikov Research Institute for Vaccines and Sera, Moscow, Russia*

According to the 16 edition of the Novel Coronavirus Diagnosis and Treatment Plan issued by the Ministry of Health of Russia, options for antiviral therapy include umifenovir and interferon -alpha-2b . Umifenovir is an indole-derivative molecule, that blocks viral fusion. Interferon alpha (IFN-α) is a glycoprotein with antitumor, antiviral, and immunomodulatory activity. Both drugs are broad-spectrum antivirals that licenced for treatment and prophylaxis of respiratory infections in Russia. We assessed the in vitro antiviral activity of umifenovir and interferon-alpha-2b against the ancestral Wuhan-like SARS-CoV2 (Dubrovka strain), and the variants of concern (VOCs) Delta (Podolsk strain) and Omicron (Otradnoe strain). Vero CCL81 cells were pre-treated 2 hours with different concentrations of the compounds

before infection. Antiviral activity against the SARS-CoV-2 viruses was determined using MTT cell viability assay for Dubrovka strain and cell-ELISA viral replication assay for Podolsk and Otradnoe strains. Both drugs have equipotent antiviral activity against the Wuhan-like and the VOCs viruses. IC50 have ranged from 3 μg mL-1 to 15, 5 μg mL-1 for umifenovir and from 10.2±1.5 to 14.5±5.5 IU mL-1 for interferon-alpha-2b. Umifenovir and interferon-alpha-2b with broad spectrum activity against SARS-CoV2 variants and other respiratory viruses featuring similar symptoms could be extremely beneficial to combat infections. This makes it suitable as the drugs that have no requirements for a diagnostic test to be performed before administration.

562. USC-026 and USC-089a Suppress the Replication of SARS-CoV-2 in the Lungs of Syrian Hamsters and Mitigate Viral Pathogenesis More Effectively Than Remdesivir

Charles E. McKenna, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Nicholas A. Lentini, *University of Southern California, Department of Chemistry, CA, United States*
Justin M. Overhulse, B.S., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Samantha B. Riemann, B.S., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Boris A. Kashemirov, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Anna Cline-Smith, *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Ann E. Tollefson, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Karoly Toth, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*

The diminished protection of the existing vaccines against emerging novel variants of SARS-CoV-2 accentuates the need for effective antiviral compounds to prevent and treat serious illness caused by the virus. We have been developing alternative prodrugs of GS-441524, the parent nucleoside component of remdesivir, a repurposed antiviral currently used against acute SARS-CoV-2 in the clinic, albeit with at best modest efficacy. Here, we report proof of concept that our prodrug approach can also be applied to GS-441524 to give novel pronucleotides that are effective against SARS-CoV-2 infection in Syrian hamsters.

In vitro inhibition of SARS-CoV-2 infection was assayed on Vero E6 cells by immunostaining infected cells, which were then quantified by an EliSpot plate reader. Two compounds, USC-026 and USC-89a had 6- and 9-fold lower EC50s than remdesivir; these compounds were chosen for in vivo evaluation.

Syrian hamsters, which are permissive for the replication of SARS-CoV-2, were infected intranasally with the virus and treated with daily intraperitoneal injections of 30 mg/kg of the compounds. Both USC-026 and USC-089a significantly suppressed the replication of SARS-CoV-2 in the lungs of hamsters, while remdesivir had no effect at the molar equivalent dose. Further, treatment with either compound significantly reduced body weight loss of the infected hamsters. In line with the remdesivir's inability to significantly suppress virus replication, the mitigation of pathology was less pronounced for this compound.

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563. Use of Deep Learning In Design of New Antiviral Candidates Targeting Both Wild and Mutant Influenza A Virus

Hovakim Zakaryan, Ph.D., *Denovo Sciences Inc, Yerevan, Armenia*
Mher Matevosyan, M.S., *Denovo Sciences Inc, Yerevan, Armenia*
Vardan Harutyunyan, M.S., *Denovo Sciences Inc, Yerevan, Armenia*
Hamlet Khachatryan, Ph.D. Seeking, *Denovo Sciences Inc, Yerevan, Armenia*

Influenza virus remains a major public health challenge due to its high morbidity and mortality and seasonal surge. Although antiviral drugs against the influenza virus are widely used as a first-line defense, the virus undergoes rapid genetic changes, resulting in the emergence of drug-resistant strains. Thus, new antiviral drugs that can outwit resistant

strains are of significant importance. Herein, we used deep reinforcement learning (RL) algorithm to design new chemical entities (NCEs) that are able to bind to the native and H275Y mutant (oseltamivir-resistant) neuraminidases (NAs) of influenza A virus with better binding properties than oseltamivir. We generated more than 60000 NCEs, which were prioritized based on the filtering rules, structural alerts, and synthetic accessibility. Then, 18 NCEs with better MM/PBSA scores than oseltamivir were further analyzed in molecular dynamics (MD) simulations. The MD experiments showed that 8 NCEs are able to form very stable complexes with the binding pocket of both native and H275Y mutant NAs of H1N1. Furthermore, most NCEs demonstrated much better binding affinity to group 2 (N2, N3, and N9) and influenza B virus NAs than oseltamivir, suggesting that our RL-generated chemical structures could be considered as antiviral candidates against influenza A and B viruses. Based on obtained results, we assume that our RL-based technology could be successfully used to design small drug-like molecules targeting both native and mutated proteins, thereby accelerating the discovery of drug candidates against drug-resistant viruses.

564. β-D-N4-Hydroxycytidine (NHC), Active Ribonucleoside Analog of Molnupiravir, Impairs Viral RNA Synthesis and Recombination of SARS-CoV-2, MERS-CoV, and MHV

Laura Stevens, M.S., *Vanderbilt University Medical Center, Nashville, TN, United States*
Jennifer Gribble, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Andrea Pruijssers, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Maria Agostini, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Jordan Anderson-Daniels, Ph.D., *Vanderbilt University Medical Center, Nashville, TN, United States*
Tia Hughes, M.S., *Vanderbilt University Medical Center, Nashville, TN, United States*
Amy Sims, Ph.D., *United States*
Timothy Sheahan, Ph.D., *United States*
George R. Painter, Ph.D., *Emory Institute for Drug Development, United States*
Ralph Baric, Ph.D., *United States*
Andrew Routh, Ph.D., *United States*
Mark Denison, M.D., *Vanderbilt University Medical Center, Nashville, TN, United States*

Nucleoside analog β-D-N4-hydroxycytidine (NHC, EIDD-1931) is the parent compound of molnupiravir (MOV, MK-4482, EIDD-2801), the orally bioavailable prodrug that is authorized or approved in many countries for treatment of mild to moderate COVID-19 in individuals at high risk for disease progression. The demonstrated increase in transition mutations in the presence of NHC/MOV during Coronavirus (CoV) replication in vitro and in vivo is the basis for a mechanism of action of lethal mutagenesis leading to population extinction. However, during single rounds of replication in the presence of low concentrations of NHC, immediate high-level impairment of replication occurs when only low frequency transition mutations are observed. This suggests additional or alternative mechanisms of action for NHC. We here show for the beta-CoVs SARS-CoV-2, MERS-CoV, and mouse hepatitis virus (MHV) that NHC treatment results in a dose-dependent decrease in subgenomic mRNA abundance, an increase in the production and packaging of defective viral genomes, and a decrease in the specific infectivity (genomes per plaque forming units) of released virus particles. Our results are consistent with NHC impairing viral RNA synthesis and recombination, supporting a mechanism of antiviral activity by lethal defection in addition to probable lethal mutagenesis at high concentrations over multiple replication cycles. These data highlight the importance of testing of all nucleoside analog inhibitors, including mutagens, for impacts on viral replicase functions, recombination, and viral RNA synthesis.

566V. Comparison of SARS-CoV-2 Variants in a Transgenic hACE2-Mouse Model

E. Bart Tarbet, Ph.D., *Utah State University, Logan, Utah, United States*
Ashley N. Sheesley, B.S., *Utah State University, Utah, United States*
Sydney Worley, B.S., *Utah State University, Logan, Utah, United States*
Scott A. Gibson, M.S., *Utah State University, Logan, Utah, United States*
Arnaud J. Van Wettere, D.V.M., Ph.D., *Utah State University, Logan, Utah, United States*
Brett L Hurst, Ph.D., *Utah State University, Logan, Utah, United States*

SARS-CoV-2 continues to mutate, producing variants that may have increased transmissibility, disease severity, and/or be less susceptible to public health control measures. As the virus changes, animal models for COVID-19 may also need to change to match circulating variants. These studies compared major variants of SARS-CoV-2 against the index virus in a transgenic hACE2-mouse model. To evaluate the variants, the LD90 was determined for the index (10^3.2), Alpha (10^2.6), Beta (10^2.9), Gamma (10^1.0), Delta (10^1.7), and Epsilon (10^4.0) viruses. The LD90 for Omicron

could not be determined as it was non-lethal. Weight loss, mortality, viral load, cytokine responses, and histological changes in vivo were evaluated. Beta and Delta lost significant weight after challenge, while Omicron showed the least weight loss. On day 3 p.i., Omicron had >1 log reduction in lung virus titers, while on day 6 p.i., Beta, Gamma, and Omicron showed >2 log reduction in titers. Of note is the trend toward a later mean day of death (MDD) from 7.1 to 10.2 days for mice that died as new variants emerged. In addition, Omicron showed the lowest virus titers on day 6 p.i., but significantly higher IL-4, IL-12, and IL-17 in lung lavage. Although not statistically significant due to the non-parametric analyses required to analyze clinical score data, a trend toward a later onset of clinical signs was observed for newer variants. Considering the variants as they emerged chronologically, this model suggests that clinical scores and MDD are observed at progressively later time points, lung virus titers decrease, and for Omicron, higher cytokines are observed in lung lavage.

568V. Impact of Baloxavir-resistant Influenza Virus Mutants On Viral Growth and Drug Susceptibility

Brady Hickerson, Ph.D., *FDA, Silver Spring, Maryland, United States*
Svetlana Petrovskaya, Ph.D., *FDA, Silver Spring, Maryland, United States*
Harold Dickensheets, Ph.D., *FDA, Silver Spring, Maryland, United States*
Raymond Donnelly, Ph.D., *FDA, Silver Spring, Maryland, United States*
William Ince, Ph.D., *FDA, Silver Spring, Maryland, United States*
Natalia Ilyushina, Ph.D., *FDA, Silver Spring, Maryland, United States*

Influenza A and B viruses (IAV and IBV) can cause acute respiratory disease is humans. Baloxavir marboxil (baloxavir) is an FDA-approved inhibitor of the polymerase acid (PA) protein endonuclease function. The IAV PA I38L, I38T, and E199D and IBV PA I38T substitutions were recently found to confer baloxavir resistance. However, the impact of these substitutions on viral fitness and drug susceptibility when present in mixed viral population is unknown. Here, we generated recombinant A/California/04/09 (H1N1)-like viruses with PA I38L, I38T, or E199D substitutions and B/Victoria/504/2000-like virus with PA I38T substitution and then prepared a panel of mixtures containing ratios of the wild-type (WT) and mutant (MUT) viruses. We then assessed the replication activity and baloxavir susceptibility of the WT:MUT mixtures in normal human bronchial epithelial (NHBE) cells. Droplet-digital PCR (ddPCR) and next-generation sequencing (NGS) were used to verify and track WT:MUT ratios. We found that the IAV PA I38T MUT at any ratio did not alter replication kinetics. However, presence of the IAV PA I38L, E199D, and IBV PA I38T MUTs at ≥75% or ≥90% significantly altered polymerase activity (1.3-fold) or decreased viral titers at 72 hours post-infection (1.2-fold), respectively. Furthermore, only 13% of the PA I38T MUT was needed to significantly reduce baloxavir sensitivity of the H1N1 virus in NHBE cells. We also found that WTs outcompeted MUTs in NHBE cells when initial mixtures contained ≥50% of the WT viruses. Understanding the impact of baloxavir-resistant substitutions on viral fitness and drug susceptibility is needed to maintain the clinical effectiveness of baloxavir.

569V. Novel Formulations of Remdesivir as Organic Salts and Ionic Liquids (OSILs) Show Improved in vitro Antiviral Activity Against SARS-CoV-2

Vanessa Correia, Ph.D., *National Health Institute Dr. Ricardo Jorge (INSA), Lisbon, Portugal*
Miguel M. Santos, Ph.D., *LAQV-REQUIMTE, Chemistry Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal*
Luís A. Santos, Ph.D., *National Health Institute Dr. Ricardo Jorge (INSA), Lisbon, Portugal*
Francisco Faísca, *LAQV-REQUIMTE, Chemistry Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal*
Luís C. Branco, Ph.D., *LAQV-REQUIMTE, Chemistry Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal*
Helena Rebelo-de-Andrade, Ph.D., *National Health Institute Dr. Ricardo Jorge (INSA); Host-Pathogen Interactions Unit, Research Institute for Medicines, iMed-ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal*

The development of more effective antiviral molecules against SARS-CoV-2 is urgently needed and a global health priority. In recent years, promising novel formulations of active pharmaceutical ingredients have been achieved by combination with Organic Salts and Ionic Liquids (OSILs). Here, we set out to explore the benefits of the OSILs approach with Remdesivir (RDV), a molecule originally developed to treat Ebola and the only FDA-approved direct-acting antiviral for COVID-19, in inhibiting SARS-CoV-2 replication in vitro. Five investigational RDV-OSILs - [RDVH]

[C1SO3], [RDVH][C3SO3], [RDVH][C6SO3], [RDVH][C9SO3], and [RDVH][TSA]; were studied, together with RDV and the respective counter-ions. In vitro experiments were performed in VeroE6 cells, using the USA-WA1/2020 reference strain. CellTiter-Glo Luminescent Cell Viability Assay was used to assess cytotoxicity and antiviral activity, with the latter being further assessed by real-time qRT-PCR and TCID50 assay. All RDV-OSILs displayed a similar cytotoxic profile to the original molecule (CC50 356.83->400μM). [RDVH][C3SO3] and [RDVH][C9SO3] exhibited improved antiviral activity as regards to its capacity to inhibit vRNA transcription (EC50 4.60 and 3.66μM) and the production of infectious progeny (5.81 and 6.25μM), compared to RDV (5.99μM/8.74μM). [RDVH][C3SO3] also exhibited improved activity in respect of its capacity to inhibit virus-induced CPE (EC50 9.42μM; RDV 12.42μM). These two activity-improved RDV-OSILs may be very promising lead candidates for the development of new and more highly effective formulations of RDV. Further research in human cells and animal models is needed to validate the results here obtained.

570V. Substituted Phenyl Ethynyl Pyridine Carboxamides As Potent Inhibitors of SARS-CoV-2 Virus

Corinne Augelli-Szafran, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Omar Moukha-Chafiq, Ph.D., *Southern Research, Alabama, United States*
John P. Tillotson, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Jaden Cowana, M.S., *Southern Research, Birmingham, Alabama, United States*
Ahmed Kaleem, *University of Alabama, Birmingham, Alabama, United States*
Shilpa Dutta, *Southern Research, Birmingham, Alabama, United States*
Sarath Sarngadharana, *Southern Research, Birmingham, Alabama, United States*
Sixue Zhang, Ph.D., *Southern Research, Birmingham, Alabama, United States*
Dahlia Cantor, B.S., *Southern Research, Birmingham, Alabama, United States*
Marina Yatchang, Ph.D., *Southern Research, Birmingham, Alabama, United States*

To date, over 635 million cases of SARS-CoV-2 have been confirmed in humans and resulted in more than 6.61 million deaths. Thus, there remains an urgent need for potent therapies that can mitigate coronavirus infections. Through our antiviral medicinal chemistry efforts, we identified a novel phenyl ethynyl pyridine carboxamide, SRI-45975, that exhibits antiviral activity in the low micromolar range (EC50 = 0.99 μM) against SARSCoV- 2 using human alveolar (A549-ACE2) cells, and also shows a viral titer reduction of 3.2 log units at 9 μM. Furthermore, in human airway epithelial cultures, SRI-45975 lowered replication below the limit of detection. This compound shows acceptable in vitro metabolic stability (t1/2 > 58 min), but has low solubility [solubility = 3.4 μM in simulated intestinal fluid (SIF) at pH 7.4]. Our structure-activity relationship (SAR) studies resulted in the identification of a second novel compound, SRI-46488, which has a four-fold improvement in solubility (solubility = 17 μM) and an increase in potency against SARS-CoV-2 (EC50 = 0.084 μM) while maintaining other favorable properties such as metabolic stability, permeability and LogD. To assist SARS-CoV-2 drug discovery, in-house high throughput screening data of ~1400 compounds were used to develop a machine learning model to predict phenotypical anti-SARS-CoV-2 activity with ~87% accuracy. The SAR studies and biological results will be discussed.

571V. Susceptibility of Mammalian Cell Lines to Infection with H3N2 Influenza Virus and Differential Expression of ANP32A, an Acidic Leucine-Rich Nuclear Phosphoprotein

Babu L. Tekwani, Ph.D., *Infectious Diseases Department, Scientific Platforms, Southern Research, Birmingham, Alabama, United States*
Fahim Ahmad, Ph.D., *Infectious Diseases Department, Scientific Platforms, Southern Research, Alabama, United States*
Corinne E. Augelli-Szafran, Ph.D., *Southern Research, Birmingham, AL, United States*
Richard J. Whitley, M.D., *University of Alabama at Birmingham, Birmingham, AL, United States*

The proteins from the acidic leucine-rich nuclear phosphoprotein 32 (ANP32) family are involved in the proliferation & differentiation of cells, regulation of transcription, and export of messenger RNA. Recent studies have reported the critical role of ANP32A in the replication of the influenza virus and the upregulation of vRNA synthesis through interaction with Influenza RNA-dependent RNA-polymerase (RdRp). Several commonly used host cell lines, MDCK (Madin-Darby Canine Kidney), A549 (adenocarcinoma human alveolar basal epithelial cells), Vero (green monkey kidney epithelial cells), HeLa (human cervical cancer cells) and HEK-293 (human embryonic kidney 293 cells), were assessed for susceptibility to H3N2 influenza virus infection by evaluation of cytopathogenic effect (CPE) and expression

of viral proteins (NP and PB2) and host nucleoproteins (ANP32A). MDCK cells showed the highest CPE followed by A549 cells when compared to other cells in response H3N2 infection. Interestingly, the highest expression of viral proteins nucleoprotein (NP) and RdRp PB2 subunit was observed in A549 cells. Kinetics of H3N2 infection-induced CPE showed faster infectivity in A549 followed by Vero, HeLa and HEK-239 cells. However, CPE in these cell lines showed recovery at 72 hours post-infection. The basal expression of ANP32A was negligible in mock-infected MDCK and A549 cells. Infection with H3N2 produced a time-dependent increase in the expression of ANP32 in MDCK and A549 cells. These results suggest potential correlations between differential expression of ANP32A in the mammalian host cells and their susceptibility to infection with the influenza virus.

600. A Host Kinase Inhibitor, VKT-034, Is Highly Effective Against Vacells and Human Skin Organ Culture

Jennifer Moffat, Ph.D., *SUNY Upstate Medical University, Syracuse, New York, United States*
Megan Lloyd, Ph.D., *SUNY Upstate Medical University, New York, United States*
Dongmei Liu, *SUNY Upstate Medical University, Syracuse, New York, United States*
Michael Perfetti, *ViroKyne Therapeutics, United States*

Varicella zoster virus (VZV) is a human-restricted alphaherpesvirus that causes varicella and herpes zoster. VZV vaccines are effective, but infections are still common. Antiviral therapy with acyclovir (valaciclovir prodrug) or penciclovir (famciclovir prodrug) is moderately effective if given early, but resistance and toxicity may occur. There is a need for new antivirals that are safe and effective, and that may synergize with acyclovir. Here, we evaluated VKT-034, a cellular kinase inhibitor, for its effectiveness against VZV in cells and skin organ culture. VKT-034 is a substituted indazole. In ARPE-19 cells, VKT-034 prevented VZV-ORF57-Luc spread (EC50 of 1.53 μM) and was not toxic (CC50 of 90.91 μM). It was unknown if VKT-034 was effective against VZV in a biologically relevant human skin model. To address this, we obtained adult human skin from reduction mammoplasties and cut it into 1-cm2 pieces. Skin explants were inoculated with VZV-ORF57-Luc by scarification and placed on NetWells™ at the air-media interface. At 2 hpi, VKT-034 was added to the media at 5, 10, or 20 μM. VZV spread was measured by bioluminescence on days 1, 3, 5, and 7. VKT-034 significantly reduced VZV spread at 10 and 20 μM in a dose-dependent manner, and the high concentration was superior to the positive control, cidofovir (10 μM). Future studies will address the synergy with acyclovir, time-of-addition studies, toxicity in skin, and whether VKT-034 is effective in a humanized mouse model (NuSkin model).

601. Accumulation of Mutations in BKPyV Genome In Kidney Transplant Recipients Treated with Cidofovir

Olga Mineeva-Sangwo, Ph.D., *Department of Microbiology, Immunology and Transplantation, Katholieke Universiteit Leuven, Leuven, Belgium*
Elisabet Van Loon, *KU Leuven, Leuven, Belgium*
Graciela Andrei, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Maarten Naesens, *KU Leuven, Leuven, Belgium*
Robert Snoeck, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

BK polyomavirus (BKPyV) is a human DNA virus with a seroprevalence ranging from 55% to 90%. In kidney transplant recipients, BKPyV infection can lead to nephropathy and even graft loss. Current recommendation for treating BKPyV infections is to reduce immunosuppression. In addition, fluoroquinolones, leflunomide, cidovofir (CDV), and intravenous immunoglobulins are often used as adjunctive therapy. However, some patients may still show signs of BKPyV infection despite adjustments. It has been suggested that in kidney recipients genetic characteristics of BKPyV may play a role in treatment outcome. We analyzed BKPyV DNA sequences obtained in samples from kidney recipients with viremia (n = 32) collected at two time points: three months and at least one year after transplantation. Twelve patients (37.5%) had received CDV therapy during the studied period, which lasted an average of 66 days. We compared, where possible, the BKPyV non-coding control region (NCCR) and the entire coding region at both time points for each patient. NCCR comparison showed mutations in urine samples for five (5/27; 18.5%) patients and in plasma samples for four (4/8; 50%) patients. Analysis of the entire coding region showed amino acid changes in urine samples for nine (9/26; 34.6%) patients and for three (3/8; 37.5%) patients in plasma samples. In CDV-treated patients, viral mutations were observed in urine samples for one patient and for two patients in plasma samples. Our study showed that accumulation of mutations in BKPyV genome can occur over time in kidney transplant recipients with viremia independently from CDV treatment. Mutation frequency was lower in patients treated with CDV.

602. An HSV-1 DNA Polymerase Multidrug Resistance Mutation Identified in an HSCT Recipient Confirmed by CRISPR/Cas9-mediated Gene Editing

Hanna H. Schalkwijk, *Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Aspasia Georgala, *Jules Bordet Institute, Belgium*
Sarah Gillemot, *Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Arturo Temblador, *Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Dimitri Topalis, *Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium*
Sebastian Wittnebel, *Jules Bordet Institute, Brussels, Belgium*
Graciela Andrei, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Robert Snoeck, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*

Background: HSV-1 can cause severe mucocutaneous infections in immunocompromised hosts. In these patients, emergence of drug resistance mutations causes difficulties in the management of infections. Objective: To evaluate viral evolution by characterizing 17 HSV-1 isolates recovered from orofacial and anogenital lesions in a severe combined immunodeficiency (SCID) patient over 7 years' before and after hematopoietic stem cell transplantation. Methods: Spatial and temporal evolution of drug resistance was characterized genotypically [Sanger sequencing and next-generation sequencing (NGS) of viral thymidine kinase (TK) and DNA polymerase (DP)], and phenotypically (cytopathic effect reduction assay). CRISPR/Cas9 was used to introduce the novel DP Q727R mutation and dual infection competition assays were performed to assess viral replication fitness. Results: All isolates had identical genetic backgrounds suggesting that orofacial and anogenital infections derived from the same virus lineage. Eleven isolates proved heterogeneous TK virus populations by NGS, which were undetectable by Sanger sequencing. Thirteen isolates were acyclovir resistant due to TK mutations, and the DP Q727R isolate additionally exhibited foscarnet and adefovir resistance. Recombinant Q727R mutant virus, generated by CRISPR/Cas9, showed multidrug resistance and increased replication fitness under drug pressure.

Conclusion: Long-term follow-up of a SCID patient revealed virus evolution and frequent reactivation of wild-type and TK mutant strains, mostly as heterogeneous populations. The DP Q727R resistance phenotype was confirmed using CRISPR/Cas9, a useful tool to validate novel resistance mutations.

603. Broad Anti-DNA Virus Activity of Novel Cyclic Amino Acid Phoshonamidate Prodrugs of Acyclic Nucleoside Analogues

Elisabetta Groaz, Ph.D., *Rega Institute, Medicinal Chemistry, Leuven, Belgium*
Min Luo, Ph.D., *Rega Institute, Medicinal Chemistry, Belgium*
Graciela Andrei, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Robert Snoeck, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Dominique Schols, Ph.D., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Piet Herdewijn, Ph.D., *Rega Institute, Medicinal Chemistry, Leuven, Belgium*

Infections with DNA viruses are an important medical and public health problem, especially when affecting immunocompromised patients. The associated risk for zoonotic spillover and pandemic potential is also expected to rise, as demonstrated by the recent monkeypox outbreak. While the ability of some human herpesviruses to invade the central and peripheral nervous system is well recognized, increasing evidence are gradually delineating a potentially distinctive role of these viruses in the onset of severe neurological disorders, whose etiology remains poorly understood (e.g., Alzheimer's and Parkinson's disease, multiple sclerosis, epilepsy, etc.). This alertness translates directly into an urgent need for new broad-spectrum, orally bioavailable anti-DNA virus agents that can deliver a more favorable toxicity profile and clinical benefit than currently accessible therapeutic options. Herein, we describe the recent discovery of cyclic amino acid phoshonamidates as novel prodrugs of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine (HPMPA) and (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, cidofovir). These unique prodrugs chemically diverge from known phosphonoester HPMP conjugates, thereby possibly undergoing an independent activation pathway on a mechanistic level. The design was shown to tolerate quite a lot of structural variety at the

amino acid promoiety ranging from small substituents (e.g., alanine, valine, and leucine) to bulkier groups (e.g., phenylalanine), since all analogues showed excellent in vitro antiviral activity and selectivity against a wide range of DNA viruses, including herpesviruses, and adenoviruses.

604. Differences in the Pharmacokinetics of Tyrosinamide (USC-087) and Homoserinamide (USC- 093) Prodrugs of (S)-HPMPA Are Responsible for the Lower Toxicity of USC-093 in the Syrian Hamster Model of Adenovirus Infection

Karoly Toth, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Samantha B. Riemann, B.S., *University of Southern California, Department of Chemistry, CA, United States*
Justin M. Overhulse, B.S., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Mohamed Mottaleb, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Ann E. Tollefson, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Caroll B. Hartline, B.S., *University of Alabama at Birmingham, Department of Pediatrics, Birmingham, AL, United States*
Jonathan Oliva, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Jiayi Yang, *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Boris A. Kashemirov, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Francis Sverdrup, *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Scott H. James, M.D., *University of Alabama at Birmingham, Department of Pediatrics, Birmingham, AL, United States*
Charles E. McKenna, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*

Adenoviruses cause mild infections in healthy adults but can result in serious disease in immunocompromised individuals. HPMPA, an acyclic nucleoside phosphonate (ANP) has broad spectrum activity against DNA viruses but has poor bioavailability and is nephrotoxic. Previously, we reported that an N-hexadecyl tyrosinamide ester and an N-hexadecyl homoserinamide ester of HPMPA (USC-087 and USC-093, respectively) were very effective orally against HAdV-C6 in hamsters, and that USC-093 had decreased nephrotoxicity in this model. After intraperitoneal (IP) administration, USC-093 exhibited a 3-fold lower plasma exposure than USC-087 based on levels of the prodrugs and the primary metabolite HPMPA. After oral dosing, this trend was maintained with USC-093 resulting in 3-fold lower HPMPA plasma exposure than USC-087, which is surprising because the prodrugs are equally efficacious. An explanation for this may be that HPMPA exposure in the liver, the main target for adenovirus pathogenesis, is close to 4-fold higher after USC-093 IP injection. Prolonged high concentration of ANPs was shown to result in accumulation of the compounds in the kidney; indeed, USC-087 has a 6-fold higher HPMPA kidney exposure than USC-093 after IP dosing. Oral dosing of USC-087 also resulted in substantially higher kidney exposure of HPMPA versus USC-093. We believe that this increased accumulation of HPMPA in the kidney is responsible for the elevated nephrotoxicity of USC-087. These results demonstrate that changing homoserine for tyrosine as the linking amino acid ester significantly changed the pharmacokinetic profile of the compound, resulting in decreased toxicity while maintaining high efficacy.

605. Effect of Castalagin Against HSV-1 Infection In Mice

Adelina Stoyanova, Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*
Viktor Rashev, Ph.D. Seeking, *The Stephan Angeloff Institute of Microbiology, BAS, Bulgaria*
Andrey Popatanasov, Ph.D. Seeking, *Institute of Neurobiology, BAS, Sofia, Bulgaria*
Lyubka Tancheva, Ph.D., *Institute of Neurobiology, BAS, Sofia, Bulgaria*
Stephane Quideau, Ph.D., *University of Bordeaux, Bordeaux, France*
Angel S. Galabov, M.D., Ph.D., *The Stephan Angeloff Institute of Microbiology, BAS, Sofia, Bulgaria*

Herpes simplex viruses (HSVs) are ubiquitous and known since ancient times. They often infect humans, causing a number of diseases from mild uncomplicated mucosal infections to life-threatening conditions. The treatment of infections caused by HSV-1 and HSV-2, embrace a huge number of antiviral drugs. Recently, special interests as anti-herpetic agents are tannins, which are a group of polyphenols. divided into two groups of condensed and hydrolysed compounds. One of the groups of hydrolysable tannins is the ellagitannins. There is a lot of evidence in the literature that different types of ellagitannins show anti-herpesvirus activity. In this study, we test in vivo anti-herpetic effect of castalagin, an ellagitanin compound, extracted from Quercus robur, towards HSV-1 infection in newborn mice. The therapy courses with castalagin include groups receive different daily doses, 20 mg/kg, 10 mg/kg, 7.5 mg/kg or 5 mg/kg of compound. The acute toxicity determination in mice showed i.p. LD50 value of 295 mg/kg. Toxicological picture of intoxication as well prolonged toxicity was done as well. The compound manifested a marked activity at HSV-1 intracerebral infection dose of LD90/0.02 ml when administered in a 7 days course at s.c. (7.5 and 10 mg/kg). Some protection effect was recorded at 20 mg/kg. The dose of 5 mg/kg was ineffective. The reference course of acyclovir demonstrated a marked activity at daily dose of 20 mg/kg,

606. Epstein-Barr Virus-associated Posttransplant Lymphoproliferative Disorders: Providing An Inside Into the Genetics and Biology of EBV

Martyna Pociupany, Ph.D. Seeking, *KU Leuven, Leuven, Belgium*
Graciela Andrei, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Belgium*
Robert Snoeck, Ph.D., *Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium*
Daan Dierickx, M.D., Ph.D., *Belgium*

Epstein-Barr virus (EBV)-related post-transplant lymphoproliferative disorder (PTLD) is a rare but life-threatening complication of both hemopoietic stem cell (HSCT) and solid organ transplantation (SOT). This disorder is caused by drug-induced reduced immune surveillance of the immune system leading to uncontrolled proliferation of lymphocytes driven, in most cases, by the oncogenic EBV. This γ-herpesvirus is a common virus that usually does not cause any serious clinical consequences in healthy individuals but is associated with the majority of PTLD cases. However, recently it was reported that even up to 50% of PTLD cases are not related to EBV. Thus, researchers question over the mechanisms that contribute to the development of PTLD in EBV-negative cases. Therefore, we aim to analyze viral co-infections with different DNA viruses and the exosome cargo in plasma samples recovered at 3 different timepoints [at the time of transplantation (T0), and at 3 months (T1) and 6 months (T2) post-hematopoietic stem cell transplantation (HSCT). The following cohorts will be included: 24 patients who developed PTLD [HSCT-PTLD(+)] and 50 patients that did not [HSCT-PTLD(-)]. Our primary results indicate that at all the three timepoints, the EBV microRNA's BART-12, BART-16, BART-2-5p and the cellular miRNA-23-3p are downregulated and the miRNA-106-5p is upregulated in HSCT-PTLD(+) patients compared to HSCT-PTLD(-). Whereas the cellular microRNA's miRNA-21-5p and miRNA-19a-3p are upregulated only at T1 and downregulated at T0 and T2. Our investigations will help with filling the knowledge gap in the understanding of PTLD.

607. Filociclovir is a Potent Inhibitor of Human Adenovirus F41 and a Strong Candidate for Treating Pediatric Adenovirus Infections

Islam Hussein, Ph.D., *Microbiotix, Worcester, United States*
Ann E. Tollefson, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, Missouri, United States*
Karoly Toth, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, Missouri, United States*
Adriana Kajon, *United States*
Terry Bowlin, *United States*

Recently, clusters of acute non HepA-E hepatitis cases in previously healthy children have been reported globally. At least, 1010 cases were identified in 35 countries, 5% of those cases required liver transplantation and 2% died. The exact cause is not yet known, but there is circumstantial evidence suggesting that human adenovirus F41 (HAdV-F41) infection might be playing a role. Beyond its role as a causative agent of gastroenteritis, HAdV-F41 has also been recently detected in cases of disseminated disease in transplant recipients. No antiviral drug has been approved for treating human adenovirus infections. Here, we show that filociclovir (FCV), a nucleoside analog, is a potent inhibitor of HAdV-F41 in cell culture using 2 approaches, namely immunostaining of infected cells and virus yield reduction assay. The activity of FCV was compared to 3 other known antivirals: cidofovir (CDV), ganciclovir (GCV) and valganciclovir

(VGCV). Among the 4 compounds examined in this study, FCV was the most potent, with an EC50 of 3.5 μM. These compounds can be ranked by potency as follows: FCV > CDV > GCV ≥ VGCV. In addition, FCV was 10-fold more potent than CDV in a virus yield reduction assay. This report provides timely and valuable methodologies to the research community for testing antivirals against HAdV-F41. Our findings also support the continued development of FCV for various therapeutic applications, including pediatric hepatitis, if a causal relationship is firmly established in the future. Furthermore, we are also testing the in vitro activity of FCV against a panel of HAdV clinical isolates commonly associated with pediatric infections.

608. HSV-1 Chromatin is Enriched in Highly Dynamic Histone Variant H2A.B in Replicating and Transcribed Viral DNA

Arryn Owens, B.S. Seeking, *Cornell University, Ithaca, NY, United States*
Esteban Flores Cortes, Ph.D. Seeking, *Cornell University, NY, United States*

Herpes Simplex Virus 1 (HSV-1) is a prevalent human virus, infecting about 70% of people. Though infection often causes just cold sores, it can progress to severe presentations. Pathogenesis relies on lifelong infection with periodic reactivations that also promote viral transmission. There are no curative treatments for HSV-1, partly because the regulation of the transitions in viral gene expression between lytic and latent infections remains unclear. Most current models suggest that epigenetic regulation plays a major role, and epigenetic modulators have been proposed to prevent reactivation. Lytic HSV-1 DNA is assembled in unstable nucleosomes and forms unusually dynamic chromatin, while latent viral chromatin is far less dynamic. The mechanisms of these chromatin dynamics are unknown, but histone variants associated with DNA silencing are enriched in latent viral chromatin. Histone H2A is the most variable core histone; H2A.B assembles highly dynamic chromatin and interacts preferentially with HSV-1 DNA. We evaluated the co-localization of histones H2A, macroH2A, H2A.B and H2B with replicating HSV-1 DNA using engineered cell lines expressing a tagged histone variant. Replicating viral DNA was labelled with ethynyl-modified nucleosides (EdU and EdC) and detected by click chemistry. Cells were processed by immunofluorescence and analyzed by confocal microscopy. Only H2A.B preferentially co-localized with replicating viral DNA, supporting a model in which H2A.B assembles highly dynamic viral chromatin and plays an activating role in infection. This type of epigenetic regulation is not sensitive to the epigenetic modulators commonly proposed as potential antivirals.

609. The Mechanism of Action of Filociclovir against Human Adenovirus is Different and Distinct from its Action against Human Herpes Viruses

Sydney Blackmer, B.S., *Drake University College of Pharmacy and Health Sciences, Des Moines, IA, United States*
Rebecca Grenz, B.S., *Drake University College of Pharmacy and Health Sciences, IA, United States*
Marie Nguyen, Ph.D., *Des Moines University College of Osteopathic Medicine, Des Moines, IA, United States*
Shauna Kaplan, Ph.D., *Drake University College of Arts and Sciences, Des Moines, IA, United States*
Terry Bowlin, Ph.D., *Microbiotix Inc., Worcester, MA, United States*
John Drach, Ph.D., *University of Michigan School of Dentistry, Ann Arbor, MI, United States*
Brian Gentry, Ph.D., *Drake University College of Pharmacy and Health Sciences, Des Moines, IA, United States*

The human herpes viruses (HHV) and human adenoviruses (HAdV) can cause life-threatening disease in the immunologically immature and immunocompromised populations. Current treatment options for these viruses include ganciclovir/valganciclovir and cidofovir. However, these therapeutics suffer from significant incidences of adverse effects (neutropenia and/or nephrotoxicity). Filociclovir (FCV), a methylenecyclopropane nucleoside analog (MCPNA), has previously demonstrated activity against beta HHV and HAdV with little to no observed toxicities in vivo. The mechanism of action against beta HHV is monophosphorylation by virus-encoded kinases [UL97 from cytomegalovirus (CMV) or U69 from HHV-6] followed by conversion to a triphosphate by an endogenous cellular kinase (guanylate kinase). The result is an active compound that directly inhibits the virus-encoded DNA polymerase (IC50 = 12 μM) and/or causes premature chain termination. The presence of FCV-triphosphate (121 +/- 11 pmol/10^6 cells at 120 hrs) in CMV-infected cells (MOI > 3) incubated with 5xEC50 FCV corroborates this mechanism. However, there was no triphosphate detected in HAdV-5-infected cells (MOI > 3) incubated with 5xEC50 FCV at 48 hours (limit of detection – 0.1 pmol/10^6 cells) indicating that the antiviral effect is via a different mechanism of action. In addition, multiple other MCPNAs chemically similar to FCV that are incapable of intracellular phosphorylation demonstrated activity against HAdV (EC50's = 0.01 - 21.5 μM). We therefore hypothesize a very unique paradigm - that the mechanism of action of FCV against HAdV is different and distinct from the mechanism of action against beta human herpes viruses.

610. USC-150, A Homoserinamide Oral Prodrug of Cidofovir, Prevents Lethal HAdV-C6 Infection in a Humanized Immunosuppressed Syrian Hamster Model

Samantha B. Riemann, B.S., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Boris A. Kashemirov, Ph.D., *University of Southern California, Department of Chemistry, CA, United States*
Caroll B. Hartline, B.S., *University of Alabama at Birmingham, Department of Pediatrics, Birmingham, AL, United States*
Anna Cline-Smith, *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Ann E. Tollefson, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Scott H. James, M.D., *University of Alabama at Birmingham, Department of Pediatrics, Birmingham, AL, United States*
Karoly Toth, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Charles E. McKenna, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*

Human Adenovirus (HAdV) can cause fatal infections in young children, immunocompromised patients and and the elderly. There are currently no antiviral agents approved to treat HAdV infections. Cidofovir (CDV), an acyclic nucleoside monophosphate (ANP) is used off label in severe cases but due primarily to its polar phosphonic acid group has poor bioavailability, requiring i.v. administration.

We previously reported that USC-093, an N-hexadecyl homoserinamide ester of (S)-HPMPA (the adenine analogue of CDV), showed comparable oral efficacy to USC-087 in preventing mortality against lethal HadV-C6 infection in a permissive immunosuppressed Syrian hamster model, while exhibiting reduced nephrotoxicity. Because CDV, unlike HPMPA, is utilized clinically, we have now evaluated the N-hexadecylamido homoserine ester of CDV (USC-150), which potently inhibits HAdV-C6 in cell culture (EC50 10 nm), for efficacy and toxicity in the same in vivo model.

Immunosuppressed Syrian hamsters were dosed orally with 0.6, 1.7 or 5 mg/kg USC-150 daily starting one day before intravenous challenge with an LD90 inoculation with HAdV-C6. At 0.6 mg/kg, USC-150 increased survival to 50% and was completely protective at the higher doses, which also prevented virus-induced liver damage from elevated serum bilirubin levels. Virus burden in the liver was reduced by up to 3-4 logs at 1.7 mg/kg and was undetectable at 5 mg/ kg, equivalent to an i.v. dose of 3x20 mg/kg/wk of CDV.

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611. USC-374, A Novel Prodrug of HPMPA, Suppresses the Replication of Human Adenoviruses In vitro and in vivo and Protects Hamsters from Lethal HAdV-C6 Infection

Charles E. McKenna, Ph.D., *University of Southern California, Department of Chemistry, Los Angeles, CA, United States*
Justin M. Overhulse, B.S., *University of Southern California, Department of Chemistry, CA, United States*
Caroll B. Hartline, B.S., *University of Alabama at Birmingham, Department of Pediatrics, Birmingham, AL, United States*
Anna Cline-Smith, *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Ann E. Tollefson, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*
Scott H. James, M.D., *University of Alabama at Birmingham, Department of Pediatrics, Birmingham, AL, United States*
Karoly Toth, Ph.D., *Saint Louis University School of Medicine, Department of Molecular Microbiology and Immunology, St. Louis, MO, United States*

Most human adenovirus (HAdV) infections cause mild, self-resolving illnesses. However, they can cause high morbidity and case fatality rate in immunocompromised patients. Further, emerging types, e.g. HAdV-F41 and HAdV-B14, are implicated in causing disease in otherwise healthy people. To meet the medical need for a drug to specifically treat HAdV infections, we have been developing a family of prodrugs of acyclic nucleotide phosphonate (ANP) antivirals that much more potent, have better bioavailability and exhibit lower toxicity than the parent compounds. Here, we report in vitro and in vivo efficacy data for USC-374, an (S)-HPMPA derivative equipped with a novel serinamide promoiety designed to provide enhanced aqueous solubility while retaining useful oral bioavailability and high antiviral potency (EC50 20 nm for HAdV-C6, and using a different methodology, 90 nM for HAdV-F41 vs the reference ANP antiviral cidofovir (CDV) which has a reported EC50 of 18 mM.

After intravenous challenge with the LD90 innoculation of HAdV-C6, daily oral treatments with 0.6 mg/kg USC-374 increased median survival of Syrian hamsters from 4.5 days to 7 days and decreased mortality to ~50%. At a dose of 1.7 or 5 mg/kg q.d., USC-374 protected completely against mortality and the hamsters recovered fully by 13 days. At 5 days, virus burden in the liver was signficantly lowered for hamsters treated with 0.6 or 1.7 mg/kg of USC-374 and was undetectable for the hamsters treated with 5 mg/kg of USC-374. At this time point, liver damage was also significantly reduced for the groups treated with 1.7 or 5 mg/kg of USC-374.

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612V. Characterization of Novel, Potential Antiviral Resistance Mutations in the Monkeypox Virus Replication Complex in the 2022 Outbreak

Benjamin Liu, M.D., Ph.D., *Children's National Hospital, George Washington University, Washington, DC, United States*

Background: since May 2022, a rapidly spreading monkeypox outbreak has evolved into a global health emergency, with >50,000 cases worldwide. Though vaccines may reduce morbidity/mortality, post vaccination breakthrough infections have been reported, suggestive of the circulation of immune-escape variants. Antivirals, e.g., Cidofovir (CDV) that inhibits viral DNA replication, are available for monkeypox virus (MPXV) treatment. However, the evolution of drug resistant variants is elusive. This study aimed to examine frequency and location of the mutations in the MPXV DNA replication complex (RC) and infer their impact on the antivirals. Methods: 230 MPXV genomic sequences were retrieved from the NCBI nucleotide sequence database. Among them 120 were the 2022 outbreak strains and the rest were earlier isolates. Mutations in the RC (encoded by F8L) were identified. Their potential impact on CDV was evaluated using structures of related viral and eukaryotic proteins, and structure prediction method AlphaFold. Results: classic CDV resistance mutations were not found at A314 or A684 among all MPXV sequences. Five types of novel mutations in RC were identified from 11 sequences, e.g. R25Q (2/230), E45K (2/230) and F108L (2/230). These mutations were confirmed not to exist before 2022. Topologically F108 is responsible for ensuring DNA binding affinity of the RC, whereas F108L may change the fidelity and processivity of RC, thereby compromising its sensitivity to nucleoside inhibitors. Conclusion: novel, potential drug resistant mutations were identified in the MPXV RC in the 2022 outbreak. Genomic surveillance will be warranted to monitor the evolution of resistant MPXV variants.

613. Infectivity and Replication Inhibition Effect of 5-fluorouracil on Herpes simplex virus type-1 Associated with Mutations in Thymidine Kinase Gene

Ahmed Eisa Elhag Ibrahim, D.V.M., Ph.D., *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
Mehmet Hakan Taskin, M.D., Ph.D., *Department of Medical Microbiology, Faculty of Medicine, University of Samsun, Turkey*
Cuneyt Tamer, D.V.M., Ph.D., *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
Bahadir Muftoglu, D.V.M., Ph.D., *Department of Experimental Animals, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
Hanne Nur Kurucay, D.V.M., Ph.D., *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
Seda Gozel, Ph.D. Seeking, *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*
Zafer Yazici, D.V.M., Ph.D., *Department of Virology, Faculty of Veterinary Medicine, Ondokuz Mayis University, Samsun, Turkey*

Two pandemics over the first two decades of the 21st century have shown that viruses with epidemic and pandemic potentials constitute a major threat to human health due to the lack of effective antivirals. Developing new antivirals and treatment strategies is also as important as vaccines play a crucial role in the first line of preventing viral infections. Using base analogues that directly target viruses and understanding their action mechanisms can present essential alternative treatment strategies to combat viral diseases. The current study investigated the effects of a base analogue on herpes simplex virus type-1 (HSV-1) replication in a cell culture system using the pyrimidine analogue 5-fluorouracil (FU). After that, the full-length UL-23 gene encoding thymidine kinase (TK) of HSV-1 was sequenced to detect induced mutations. The results showed a diminishing viral titer and viral load at 2 logs and 663 times, respectively, at the end of 10 consecutive passages with 5-FU. Furthermore, two mutations substitute amino acids in the non-conserved region of the TK gene, which confers drug resistance, were also identified. The current research is a feasibility study to investigate the antiviral effects of 5-FU on DNA viruses and has reinforced the fact that 5-FU can have an antiviral effect on HSV-1. However, drug resistance for viruses should not be underestimated.

614V. Novel Latency-disrupting Anti-herpesvirals & PROTACs Against KSHV

Aylin Berwanger, *Helmholtz-Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany*

The Kaposi's sarcoma-associated herpesvirus (KSHV) is a tumor-inducing herpesvirus (Human Herpesvirus HHV-8). The viral protein latency-associated nuclear antigen (LANA) is required for the latent viral replication and persistence of KSHV in the host cells. It acts via tethering the virus episome to the host nucleosomes. [1] With the aim to inhibit the essential LANA-DNA interaction, we applied a fragment-based drug design (FBDD) approach. [2] We were able to synthesize the first inhibitors and to further optimize them by using the Suzuki-Miyaura coupling. [3,4] Via Microscale Thermophoresis assay (MST) the KD value of the new hit could be determined, Electrophoretic Mobility Shift Assay (EMSA) was used to determine the inhibition. Additionally, the hit was explored regarding its ADMET properties in order to generate a new inhibitors with a decent binding affinity and a suitable in vitro pharmacokinetics (PK) profile at the same time. Our current frontrunner shows a convincingly high solubility, low LogD values, a high permeability and good metabolic stability. Furthermore, we were able to observe an antiviral effect in a KSHV replication assay. Additionally, we synthesized PROTAC molecules with the aim to degrade the viral protein LANA. The PROTACs were already tested in the first assays and show a promising affinity to the target. Furthermore, we are testing solubility, metabolic stability with liver S9 fractions and permeability of the PROTACs with Caco-2 cells.

700. Antivirally Active Dialkyl-Nucleoside Diphosphate and Triphosphate Prodrugs

Xiao Jia, Ph.D., *University of Hamburg, Hamburg, Germany*
Dominique Schols, Ph.D., *KU Leuven Rega Institute for Medical Research, Belgium*
Chris Meier, Ph.D., *University of Hamburg, Hamburg, Germany*

We report on the synthesis and evaluation of two different nucleotide prodrug systems: i) nucleoside triphosphate analogues in which the gamma-phosphate or the -phosphonate is esterified covalently by two lipophilic, non-bioreversible alkyl residues (TriPPPro-compounds); ii) nucleoside diphosphate prodrugs (DiPPro-derivatives) bearing two non-hydrolysable alkyl modification at the beta-phosphate or the beta-phosphonate. The delivery of NDP (for the TriPPPro-NTPs) and NMP (for the DiPPro-NDPs) was demonstrated in CEM cell extracts as well as in phosphate buffer, probably due to a chemical cleavage of the gamma-phosphate/phosphonate or the beta-phosphate/phosphonate moiety, respectively. In primer extension assay, we found that gamma-phosphate-modified-NTPs and NDPs were accepted as substrates by HIV-RT. Excellent anti-HIV activities of TriPPPro- and DiPPro-compounds were observed with more 40,000-fold (SI 37.500) as compared to the parent nucleoside analogue d4T.

701. C-2 Alkylated Tryptophan Derivatives, Highly Potent Entry Inhibitors of Enterovirus A71 Clinical Isolates

Ana Rosa San Felix, Ph.D., *Instituto de Química Médica, Spanish Research Council (CSIC), MADRID, Madird, Spain*
Olaia Martí-Marí, Ph.D., *Instituto de Química Médica, Spanish Research Council, Spain*
Rana Abdelnabi, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
Dominique Schols, Ph.D., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Johan Neyts, Ph.D., *Rega Institute for Medical Research, Leuven, Belgium*
María José Camarasa, Ph.D., *Instituto de Química Médica, Spanish Research Council (CSIC), Madrid, Spain*
Federico Gago, *Departamento de Ciencias Biomédica, Universidad de Alcalá, Alcala de Henares, Spain*

AL-471, the leading exponent of a class of potent HIV and enterovirus A71 (EV-A71) entry inhibitors discovered in our research group, contains four L-tryptophan (Trp) units bearing an aromatic isophthalic acid directly attached to the C2 position of each indole ring. Starting from AL-471 we (1) replaced L-Trp with D-Trp, (2) inserted a flexible linker between C2 and the isophthalic acid, and substituted a non-aromatic carboxylic acid for the terminal isophthalic acid. Truncated analogues lacking the Trp motif were also synthesized (3). Our findings indicate that the antiviral activity seems to be largely independent of the stereochemistry (L- or D-) of the Trp fragment and also that both the Trp unit and the distal isophthalic moiety are essential for antiviral activity. The most potent derivative, AL-534, with the C2 shortest alkyl urea linkage (three methylenes), showed subnanomolar potency against different EV-71 clinical isolates. This finding was only observed before with the early dendrimer prototype AL-385 (12 L-Trp units) but remained unprecedented for the reduced-size prototype AL-471. Molecular modelling showed the feasibility of high-affinity binding of the novel L-Trp-decorated branches of AL-534 to an alternative site on the VP1 protein that harbours significant sequence variation among EV-71 strains.

702. Characterization of Brazilian HIV-1 Near Full-Length Proviral Genomes from Patients under Successful First-Line Antiretroviral Therapy

Ornella Botelho, M.S., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*
Brunna Alves, Ph.D., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*
Marianne Garrido, M.D., *Hospital Federal de Ipanema, Rio de Janeiro, Brazil*
Vanusa da Hora, Ph.D., *Universidade Federal do Rio Grande, Rio Grande, Rio Grande do Sul, Brazil*
Marcelo Soares, Ph.D., *INCA, Rio de Janeiro, Brazil*

Antiretroviral therapy (ART) has revolutionized HIV treatment, increasing quality and life expectancy of people living with HIV (PLWH). However, the expansion of treatment has resulted in an increase in antiretroviral resistant viruses, which can be an obstacle to maintenance of successful ART. Thus, it is essential to understand the outcome of drug resistance mutations (DRM), including minority (low-frequency) variants, on treatment of PLHW with undetectable viral load. This study analyzed the genetic composition of HIV near full-length genome (NFLG) from archived proviruses of PLWH under successful ART; determined the presence/frequency of DRM and determined the viral subtype. Forty-six PLHIV from Rio de Janeiro (RJ) and 40 from Rio Grande (RS) had their genomic DNA extracted from peripheral whole blood. HIV NFLG was PCR-amplified and ultradeep sequenced. The presence/frequency of DRMs were analyzed in Geneious. Phylogenetic analyses were performed using PhyML and SimPlot. All samples included in the study have been sequenced and 69 (80.2%) had the HIV NFLG determined. RJ and RS showed a predominance of HIV subtypes B (78.3%) and C (67.5%), respectively. Overall, 168 DRMs were found in 63 (73.3%) samples and 105 (62.5%) of them were minority variants. Among the 168 DRMs, 68 (40.5%) were able to confer some degree of resistance to at least one drug in use by patients, yet no one showed signs of therapeutic failure. Our study contributes to the understanding of the impact of DRMs on successful therapy and supports the sustainability of combinatorial ART, since all patients maintained their successful treatment despite the high prevalence of DRMs at low or high frequency.

703. Longitudinal Evaluation of Archived HIV-1 Proviral Epitopes with High Affinity to Circulating HLA Class I Alleles as Potential Tools for an HIV Therapeutic Vaccine

Ornella Botelho, M.S., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*
Brunna Alves, Ph.D., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*
Isabel Prellwitz, Ph.D., *Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil*
Marianne Garrido, M.D., *Hospital Federal de Ipanema, Rio de Janeiro, Brazil*
Vanusa da Hora, Ph.D., *Universidade Federal do Rio Grande, Rio Grande, Rio Grande do Sul, Brazil*
Marcelo Soares, Ph.D., *INCA, Rio de Janeiro, Brazil*

Eradication of HIV is still a major challenge and a great number of people living with HIV (PLWH) are on antiretroviral therapy, who are susceptible to side effects of the lifetime therapy. Thus, novel treatment strategies are emerging based on therapeutic vaccination of PLWH, which may help control HIV replication after treatment discontinuation. The present study longitudinally evaluated the HIV-1 proviral epitopes inferred from near full-length genome (NFLG) sequences with high affinity to the most frequent HLA-A, -B and -C alleles of PLWH from Rio de Janeiro (RJ) and Rio Grande (RS). Peripheral whole blood was collected from 46 patients of RJ and 40 of RS. Genomic DNA was extracted and HIV-1 proviral NFLG were PCR-amplified and ultradeep sequenced. T-cell epitopes were predicted using the MHC-I Binding Prediction Tool. Five epitopes were selected among viral sequences from RJ (RTLNAWVKV-gag, HQKEPPFLW-pol, KHQKEPPFL-pol, TQDFWEVQL-pol and VLDVGDAYF-pol) and three from RS (KHQKEPPFL-pol, TQDFWEVQL-pol and

VLDVGDAYF-pol) that showed high affinity to HLA-A, HLA-B and HLA-C alleles carried by most patients of each region. Twenty-two PLWH from RJ had a second time point collected after two years of follow-up, and the five selected HIV-1 proviral epitopes remained in the peripheral blood compartment of all patients. Overall, a set of HIV epitopes were selected that are highly conserved among circulating viruses, with high affinity to the respective circulating HLA class I alleles, and highly stable across time in the population analyzed. We think these epitopes represent promising peptides to be used in the development of a therapeutic vaccine against HIV infection.

704. Longitudinal Evolution of HIV-1 Drug Resistance Mutations Within Proviral Quasispecies of Patients Under Successful Antiretroviral Therapy

Brunna Alves, Ph.D., *INCA, Rio de Janeiro, Rio de Janeiro, Brazil*
Ornella Botelho, M.S., *INCA, Rio de Janeiro, Brazil*
Marianne Garrido, M.D., *INCA, Rio de Janeiro, Brazil*
Marcelo Soares, Ph.D., *INCA, Rio de Janeiro, Brazil*

Increased access to antiretroviral therapy (ART) by people living with HIV (PLWH) has become a reality worldwide. In the face of the 95-95-95 challenge proposed by UNAIDS, rates of diagnosis, treatment and therapeutic success are aimed at 95%. In a context of high viral genetic variability, the clinical relevance of low-frequency variants in the intrahost viral quasispecies and the implication of the latter on the evolution of therapeutic failure are still uncertain. In this work, we aimed to determine HIV near full-length genomes (NFLG) from the archived proviral sequences and compare them longitudinally with data obtained two years later from PLWH under successful ART. HIV proviral DNA of 16 patients was extracted for PCR amplification, genomic library construction, ultradeep sequencing and analysis by reference-based assembly. A total of 16 drug resistance mutations (DRM) were found distributed in 11 samples in the first timepoint. In the second timepoint (two years later), 11 samples carried a total of 33 DRMs. Of those mutations, 26 (79%) represented low-frequency variants, and included 15 DRMs associated to the ART currently in use by the patients. Overall, the four mutations with frequency above 90% at the 1st timepoint were maintained in the 2nd timepoint, while only two low-frequency mutations remained circulating in a single patient at the 2nd timepoint. We conclude that DRMs to a single drug, even when maintained for two years in a patient, do not implicate into therapeutic failure. Moreover, low-frequency (< 50%) DRMs tend to disappear in infected PLWH and neither compromise the therapeutic success of combinatorial ART.

705. S-Acyl-Benzamide Derivatives as HIV Inactivators

Marco Robello, Ph.D., *National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, United States*
Daniel Appella, Ph.D., *National Institute of Diabetes and Digestive and Kidney Diseases, MD, United States*

Human immunodeficiency virus type 1 (HIV-1) is still a major public health concern. Highly active antiretroviral therapy (HAART) is a combination of antiretroviral drugs targeting the virus at multiple stages of its replication cycle which has helped reduce new HIV infections as well as AIDS-related deaths. However, its routine application has led to multi-drug resistance and the onset of adverse side-effects that results from long-term use. It is therefore crucial to continue the development of novel antivirals, particularly those that are inexpensive, nontoxic, and which are unlikely to result in viral resistance.

S-acyl-benzamide derivatives are chemically simple HIV inactivators targeting viral nucleocapsid protein 7 (NCp7), a 55 amino acid protein that performs essential functions during the assembly and maturation of new HIV virions. This class of molecule also shows low toxicity, and a high barrier to viral resistance. We developed a series of novel S-acyl-benzamide prodrug analogs to investigate their structure-activity relationship (SAR) profiles. Further pharmacokinetic studies were performed to elucidate the ADME properties of this novel series of derivatives.

706. Setting Up a Drug-discovery Platform for New HTLV-1 Antivirals

Clément Jacques François Heymann, Ph.D. Seeking, *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Maarten Jacquemyn, M.S., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Dirk Daelemans, Ph.D., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Dominique Schols, Ph.D., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*
Evelien Vanderlinden, Ph.D., *KU Leuven Rega Institute for Medical Research, Leuven, Belgium*

Human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus that can cause aggressive T cell leukemia or neurological disorders. Up to 10 million people are infected with HTLV-1 worldwide, but no antivirals are available yet. This project aims to discover novel inhibitors able to inhibit the transmission and/or proliferation of HTLV-1-infected cells in vitro. First, a cell-to-cell infection assay was set up. To quantify de novo HTLV-1 infection, uninfected HEK293T cells were modified by CRISPR/Cas9 to contain an enhanced green fluorescent protein (eGFP) reporter under control of the HTLV-1 5'LTR promoter. Upon infection by HTLV-1-infected C91/PL cells, the viral Tax protein will be expressed and induce eGFP expression, which is quantified by flow cytometry. The optimal ratio of the number of HEK293T reporter cells versus infected C91/PL cells showed to be 5:1, resulting in 7.38% eGFP positive cells. The assay was validated using compounds known to affect 5'LTR activity, such as rapamycin and A485, which both caused a dose-dependent reduction of eGFP expression. To facilitate screening, imaging techniques (e.g. Incucyte) are currently being implemented to assess eGFP expression. The antiviral activity will be confirmed in susceptible human dendritic cells. Infection with cell-free HTLV-1 will be evaluated by detection of integrated viral DNA using qPCR, or viral proteins using multi-parameter flow cytometry. In parallel, compounds will be evaluated for their inhibitory effect on the viability of HTLV-1-infected and uninfected cell lines. To conclude, the assays set up in this project will play a pivotal role in our aim to discover new and selective HTLV-1 antivirals.

707V. Discovery, Mechanistic Investigations and Crystallographic Study of Benzopyrimidinone-bearing Phenylalanine Derivatives as Novel HIV-1 Capsid Modulators

Shujing Xu, Ph.D., *Shandong University, Jinan, Shandong Province, China*
Lin Sun, *China*
Dang Ding, *China*
Xiaoyu Shao, *China*
Alexej Dick, *China*
Christophe Pannecouque, *China*
Xinyong Liu, Ph.D., *Shandong University, Jinan, Shandong, China*
Peng Zhan, Ph.D., *Shandong University, Jinan, Shandong, China*

The multifunctional HIV-1 capsid protein (CA) represents a highly appealing target in HIV-1 medication research. With PF74 as the lead compound, our previous efforts involved replacing the indole moiety with benzenesulfonamide piperazinone and obtained a novel HIV-1 CA modulator 11L. However, its antiviral activity and metabolic stability still need to be further improved. Herein, we obtained a series of phenylalanine derivatives containing benzopyrimidinone with improved antiviral activity and metabolic stability by cyclizing the amide bond with metabolic liabilities in 11L while retaining the privileged structure of benzenesulfonamide piperazinone, represented by compound RB-8f. Mechanism of action studies showed that RB-8f could effectively bind to the CA hexamer and interfere with the binding of host factor CPSF6 to CA, and it exhibited excellent antiviral effect with dual-stage inhibitory profile. To investigate the interaction mode between these benzopyrimidinone-bearing phenylalanine derivatives and CA hexamer, we analyzed the co-crystal structure of RB-8f. The phenylalanine core skeleton maintained the original binding mode of PF74. The carbonyl group on piperazinone formed a key hydrogen bond with Lys70. Additionally, the benzenesulfonamide moiety extended into the NTD-CTD interface, forming multiple hydrogen bonds with surrounding amino acids such as Lys70, Arg173 and Lys182. The newly introduced benzopyrimidinone group displayed a cation-π interaction with Asn53, and a hydrogen bond was built between C=O and the backbone NH of the crucial residue Thr107. Overall, these studies proved that RB-8f is a promising lead compound for further modification.

800. Strigolactones as Broad-spectrum Antivirals Against β-coronaviruses Through Targeting the Main Protease Mpro

Greta Bajetto, M.S., *University of Piemonte Orientale, Center for Translational Research on Autoimmune and Allergic Disease-CAAD,, Novara, Italy*
Matteo Biolatti, *Department of Public Health and Pediatric Sciences, University of Turin, Italy*
Marco Blangetti, *Department of Chemistry, University of Turin, Turin, Italy*
Melissa Baggieri, *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
Davide Arnodo, *Department of Chemistry, University of Turin, Turin, Italy*
Raoul Fioravanti, *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
Marco De Andrea, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Francesca Gugliesi, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*

Camilla Albano, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Selina Pasquero, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*
Ivan Visentin, *Department of Agricultural, Forestry, and Food Sciences, University of Turin, Turin, Italy*
Francesca Esposito, *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Paolo Malune, *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Matteo Bersani, *Department of Drug Science and Technology, University of Turin, Turin, Italy*
Enzo Tramontano, Ph.D., *Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy*
Cristina Prandi, *Department of Chemistry, University of Turin, Turin, Italy*
Francesca Spyrakis, *Department of Drug Science and Technology, University of Turin, Turin, Italy*
Fabio Magurano, *Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*

The current SARS-CoV-2 pandemic and the likelihood that new coronavirus strains will emerge in the immediate future point out the urgent need to identify new pan-coronavirus inhibitors. Strigolactones (SLs) are a class of plant hormones with multifaceted activities whose role in plant-related fields has been extensively explored. Recently, we proved that SLs also exert an antiviral activity toward herpesviruses, such as human cytomegalovirus (HCMV). Here we show that the synthetic SLs TH-EGO and EDOT-EGO impair β-coronavirus replication, including SARS-CoV-2 and the common cold human coronavirus HCoV-OC43. Interestingly, in-silico simulations suggest the binding of SLs in the SARS-CoV-2 main protease (Mpro) active site, and this was further confirmed by an in-vitro activity assay. Overall, our results highlight the potential efficacy of SLs as broad-spectrum antivirals against β-coronaviruses, which may provide the rationale for repurposing this class of hormones for the treatment of COVID-19 patients.

801. Novel Benzodiazepine Antivirals Selectively Active against Yellow Fever Virus with a GABAA Receptor Refractory Property

Yanming Du, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Nicky Hwang, B.S., *Baruch S. Blumberg Institute, PA, United States*
Anilkumar Karampoori, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Fang Guo, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Lin Zhang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Xuexiang Zhang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Zhengyuan Jiang, M.S., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
William Kinney, Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Ju-Tao Guo, M.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*
Jinhong Chang, M.D., Ph.D., *Baruch S. Blumberg Institute, Doylestown, PA, United States*

Yellow fever is an acute viral hemorrhagic disease that is endemic in tropical areas of Africa, Central and South America, causing an estimated 1.7 million infections and 29,000–60,000 deaths per year. An effective live attenuated yellow fever vaccine has been available since 1938, but the vaccine coverage has been insufficient and has failed to prevent outbreaks in at-risk regions. In addition, there is currently no approved treatment for yellow fever. BDAA is a benzodiazepine (BD) compound that we discovered through a high throughput screening to inhibit yellow fever virus (YFV) in cultured cells and in infected hamsters. Benzodiazepines are considered to have a privileged core structure that has been found to be effective in tranquilizer drugs by binding to the α- and γ-subunits of α- and γ-subunit containing GABAA receptors; thus, lack of binding activities to the GABAA receptors should be preferred for antivirals based on BD core. Here, we report our effort to develop benzodiazepine antivirals meeting this criterion. We analyzed BDAA and its analogs; and identified a structural variation that enables BDAA and its derivatives devoid of interactions with central and peripheral nervous system benzodiazepine receptors at 10 μM. Further structure-activity relationship studies at this position led to the discovery of novel BDAA analogs with sub-micromolar EC50s and favorable pharmacokinetic profiles.

802. Evaluation of in Vitro Selected Nirmatrelvir Resistant SARS-COV-2 in A549-ACE2 Cells

Hussin Rothan, Ph.D., *Pfizer INC, NY, United States Minor Outlying Islands*
Irina Yurgelonis, M.S., *Pfizer, New York, United States*
Patricia McMonagle, M.S., *Pfizer, NY, New York, United States*
Devendra Rai, Ph.D., *Pfizer, NY, New York, United States*
Zhenghui Li, Ph.D., *Pfizer, NY, New York, United States*

Jonathan Lee, B.S., *Pfizer, NY, New York, United States*
Li Hao, Ph.D., *Pfizer, NY, New York, United States*
Ha Nguyen, Ph.D., *Pfizer, NY, New York, United States*
Philip Lee, *Pfizer, NY, United States*
Paul Arthur Liberator, Ph.D., *Pfizer, NY, New York, United States*
Annaliesa S. Anderson, Ph.D., *Pfizer, NY, New York, United States*
Rhonda D. Cardin, Ph.D., *Pfizer, NY, New York, United States*
Yuao Zhu, Ph.D., *Pfizer, NY, New York, United States*

Nirmatrelvir is the SARS-CoV-2 main protease (Mpro) inhibitor component of PaxlovidTM. To facilitate the detection and management of antiviral drug resistance often associated with antiviral therapy, this study aims to select in vitro SARS-CoV-2 resistant mutants and determine viral fitness and sensitivity to nirmatrelvir. Infected A549-ACE2 cells were subjected to increasing concentrations of nirmatrelvir through serial passages. Plaque assay was applied for virus titration and purification, followed by virus RNA extraction, and the mutations were identified by whole genome sequencing. VeroE6 TMPRSS2 cells were used for the RT-qPCR antiviral assay. SARS-CoV-2 MproA173V and F140L+A173V mutants emerged from the drug resistance selection. High frequency of A173V mutation was observed at 1.25, 2.0 and 2.5 μM of nirmatrelvir in passages 3-7, while F140L+A173V mutation was detected in passages 6 and 7. The A173V mutant led to slightly reduced virus fitness with lower virus titer and smaller plaque sizes. The F140L+A173V mutant showed much reduced viral fitness as indicated by ~2-log reduction in virus titer as well as much smaller plaque sizes compared to the parent virus in A549-ACE2 cells. The EC50 values of the A173V mutant were similar to that of the wild-type (WT) virus. The F140L+A173V mutant demonstrated a 10-fold reduced nirmatrelvir susceptibility. Both mutants remained sensitive to remdesivir. No F140L+A173V mutation was detected, and A173V was rarely observed among >11 million SARS-CoV-2 entries in GISAID.

803. SARS-CoV-2 Nirmatrelvir Resistance Selection in VeroE6-Pgp-KO Cells

Irina Yurgelonis, M.S., *Pfizer, Pearl River, NY, New York, United States*
Hussin A. Rothan, Ph.D., *Pfizer, NY, New York, United States*
Patricia McMonagle, M.S., *Pfizer, NY, New York, United States*
Devendra Rai, Ph.D., *Pfizer, NY, New York, United States*
Zhenghui Li, Ph.D., *Pfizer, NY, New York, United States*
Li Hao, Ph.D., *Pfizer, NY, New York, United States*
Joy Yang, Ph.D., *Pfizer, Cambridge, Massachusetts, United States*
Jonathan Lee, B.S., *Pfizer, NY, New York, United States*
Ha Nguyen, Ph.D., *Pfizer, NY, New York, United States*
PaulArthur Liberator, Ph.D., *Pfizer, NY, New York, United States*
AnnaliesaS. Anderson, Ph.D., *Pfizer, NY, New York, United States*
RhondaD. Cardin, Ph.D., *Pfizer, NY, New York, United States*
Yuao Zhu, Ph.D., *Pfizer, NY, New York, United States*
Anne Moscona, M.D., *Department of Pediatrics, Center for Host-Pathogen Interaction, Department of Microbiology & Immunology, Department of Physiology & Cellular Biophysics, Columbia University Medical Center, New York, NY, United States*
Matteo Porotto, Ph.D., *Department of Pediatrics, Center for Host-Pathogen Interaction, Columbia University Medical Center, New York, NY, United States*

Nirmatrelvir is a potent and selective SARS-CoV-2 main protease (Mpro) inhibitor and the active ingredient of the oral COVID-19 treatment Paxlovid. To facilitate the detection and management of drug resistance often associated with antiviral therapy, in vitro selection of nirmatrelvir resistant SARS-CoV-2 was carried out in the presence of constant and increasing concentrations of nirmatrelvir in VeroE6-Pgp-KO cells. Selected mutant viruses were characterized by NGS, purified by plaque assay, and tested for susceptibilities to nirmatrelvir and the RdRp inhibitor remdesivir in a RT-qPCR-based assay each, respectively. Results indicated that the viral passaging yielded six unique Mpro mutation profiles: T304I, T21I+T304I, L50F+T304I, T135I+T304I, A173V+T304I, and T21I +S144A+T304I. The 6 mutant viruses showed varying degrees of reduced susceptibility to nirmatrevir in VeroE6-Pgp-KO cells, with the double mutant A173V+T304I and triple mutant T21I +S144A+T304I demonstrating EC50 increases of ~20- and 28-fold over WT each. The remainder were < 8-fold. All 6 mutants remained sensitive to remdesivir. Antiviral assay in A549-

ACE2 cells showed similar results for the mutant viruses. The results suggested that replication competent mutant virus conferring significant nirmatrelvir resistance required ≥ 2 concurrent mutations on Mpro. These identified mutations were subsequently included in the ongoing virus surveillance to support Paxlovid clinical development. At present, no A173V+T304I or T21I+S144A+T304I mutations were detected, and the rest were either not observed or at a very low frequency among > 12 million SARS-CoV-2 clinical isolates in GISAID.

804. Murine Hepatitis Virus (MHV) Mutations Selected in vitro by Nirmatrelvir, An Oral Coronavirus Main Protease (Mpro) Inhibitor

Irina Yurgelonis, M.S., *Pfizer, Pearl River, NY, New York, United States*
Hussin A. Rothan, Ph.D., *Pfizer, NY, New York, United States*
Patricia McMonagle, M.S., *Pfizer, NY, New York, United States*
Zhenghui Li, Ph.D., *Pfizer, NY, New York, United States*
PaulA rthur Liberator, Ph.D., *Pfizer, NY, New York, United States*
Devendra Rai, Ph.D., *Pfizer, NY, New York, United States*
Alexey Gribenko, Ph.D., *Pfizer, Pearl River, New York, United States*
Ha Nguyen, Ph.D., *Pfizer, NY, New York, United States*
Li Hao, Ph.D., *Pfizer, NY, New York, United States*
Annaliesa S. Anderson, Ph.D., *Pfizer, NY, New York, United States*
Rhonda D. Cardin, Ph.D., *Pfizer, NY, New York, United States*
Yuao Zhu, Ph.D., *Pfizer, NY, New York, United States*

Nirmatrelvir the antiviral component of paxlovid is a potent oral Mpro inhibitor against SARS-CoV-2 with a broad spectrum Mpro inhibition of other human coronaviruses. MHV is frequently used as a surrogate in SARS-CoV research with overall Mpro ~50% sequence identity and a high degree of conservation in their active sites. We therefore used MHV to investigate nirmatrelvir virus resistance. Murine L929 cells were infected with the MHV-A59 strain and passaged in the presence of increasing concentrations of nirmatrelvir up to 30x (25.4 μM) and 40x (33.9 μM) EC50 values. The harvested virus populations and plaque purified virus clones were sequenced to identify mutations. Growth kinetics of the mutant virus clones were evaluated using plaque assays. The susceptibilities of the mutant viruses to nirmatrelvir were tested using a RT-qPCR antiviral assay. Sequential passage of MHV in L929 cells in the presence of increasing nirmatrelvir concentrations selected P55L and S144A double mutations in the Mpro gene in all analyzed clones from 30x and 40x EC50 concentrations. Three additional mutations in Mpro, T129M, T50K, and P15A, each at <4.6% frequency, were also observed in two virus clones. The mutant viruses each showed 1-2 log reduced replication efficiency, indicating that the mutations led to reduced viral fitness. Nirmatrelvir inhibited the mutant viruses with 4.4-4.9-fold increased EC50 values over that of the parent virus. Interestingly, S144A mutation in the corresponding SARS-CoV-2 Mpro was selected in vitro against nirmatrelvir, however in combination with other mutations. The S144A mutation is rarely detected in SARS-CoV-2 clinical isolates in GISAID.

805. Nirmatrelvir, An Orally Active Mpro Inhibitor, is a Potent Inhibitor of SARS-CoV-2 Variants of Concern

DevendraK Rai, Ph.D., *Pfizer Worldwide Research, Development & Medical, Pearl River,, New York, United States*
Irina Yurgelonis, M.S., *Pfizer, New York, United States*
Patricia McMonagle, M.S., *Pfizer, NY, New York, United States*
HussinA. Rothan, Ph.D., *Pfizer, Pearl River, New York, United States*
Li Hao, Ph.D., *Pfizer, NY, New York, United States*
Alexey Gribenko, Ph.D., *Pfizer, Pearl River, New York, United States*
Elizabeth Titova, B.S., *Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, United States*
Barry Kreiswirth, Ph.D., *Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, United States*
KrisM. White, Ph.D., *Department of Microbiology, Icahn School of Medicine at Mt. Sinai, Global Health Emerging Pathogens Institute, Icahn School of Medicine at Mt. Sinai, New York, New York, United States*
Yuao Zhu, Ph.D., *Pfizer, NY, New York, United States*
AnnaliesaS. Anderson, Ph.D., *Pfizer, NY, New York, United States*
RhondaD. Cardin, Ph.D., *Pfizer, NY, New York, United States*

Emerging SARS-CoV-2 variants require prompt evaluation of vaccine and antiviral efficacy. Here, we used qRT-PCR and CellTiter-Glo (CTG) assays to evaluate the in-vitro efficacy of nirmatrelvir, the SARS-CoV-2 main protease (Mpro) inhibitor component of PAXLOVIDTM, against five SARS-CoV-2 Variants of Concern or VOC (α, β, γ, δ, ο) and two Variants of Interest or VOI, C.37 (λ) and B.1.621 (μ) in Vero E6 cells with P-glycoprotein (P-gp) gene knockout (Vero E6 P-gp KO) and Vero E6 cells overexpressing TMPRSS2 protease (Vero E6 TMPRSS2 cells). In the qPCR assay, nirmatrelvir potently inhibited the USA-WA1/2020 strain, and α, β, γ, λ, δ, μ, and ο (BA.1, BA.2, BA.2.12.1, BA.4, BA.4.6, and BA.5) variants with mean EC50 ranging between 15.9 nM and 146 nM; ο BA.2, BA.2.12.1, BA.4, BA.4.6, and BA.5 were tested in Vero E6 TMPRSS2 cells, whereas the remaining variants were tested in Vero E6 P-gp KO cells. In the CTG-assay, the EC50 ranged between 59.5 nM -171 nM in Vero E6 Pgp KO (α, β, γ, λ, and μ) and 79 - 217 nM in Vero E6 TMPRSS2 cells for the USA-WA1/2020 strain and SARS-CoV-2 variants (α, β, γ, λ, δ, and ο BA.2). Beta variant, however, exhibited reduced sensitivity (2-4-fold across assay formats) compared to USA-WA1/2020. For four δ variant isolates, the EC50 range was 111- 221 nM. Conservation of Mpro active sites of USA-WA1/2020 strain and variants tested herein likely contributed to the comparable in-vitro efficacy of nirmatrelvir.

806. The 5'UTR of HCoV-OC43 Adopts a Topologically Constrained Structure to Intrinsically Repress Translation

Barrington D. Henry Jr, Ph.D. Seeking, *Case Western Reserve University, Cleveland, OH, United States*
Matthew Mackeown, Ph.D. Seeking, *Case Western Reserve University, OH, United States*
Blanton S. Tolbert, Ph.D., *Case Western Reserve University, Cleveland, OH, United States*
Valentina Dell'Oste, *Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy*

The inception of the COVID-19 pandemic caused scientists worldwide to search for an expeditious, yet accurate characterization pipeline for viral mechanisms which cause cellular pathogenesis. Viral untranslated regions (UTRs) are integral genomic elements that contribute to such mechanisms, and are targets for therapeutic intervention. Experimental approaches are required for the generation of high-quality viral RNA structural models, which can facilitate comparative mechanistic and drug discovery efforts. Making use of a combinatorial pipeline involving experimental and computational techniques, we report the efficient characterization of conserved RNA structures within the 5'UTR of the human coronavirus OC43 (OC43) genome. Evidence is provided that the 5'UTR folds into a secondary structure with well-defined stem loops as determined by NMR spectroscopy and chemical probing. We combine experimentally determined hydrogen-bonding restraints with global structural information from SAXS to generate a 3D model in which SL1-4 adopts a topologically constrained structure whereby stem loops 3 and 4 co-axially stack. In order to evaluate the functional relevance of the SL3,4 co-axial helix, luciferase reporter constructs harboring the OC43 5'UTR were engineered to contain mutations designed to disrupt co-axial stacking. The results reveal that the SL3,4 helix intrinsically represses translation efficiency since these mutations correlate with increased luciferase expression relative to wild-type without affecting reporter mRNA levels. The work presented herein describes an efficient approach to discover a functionally relevant tertiary interaction within the OC43 5'UTR.

807. Stabilization of RNA G-quadruplexes in the SARS-CoV-2 Genome Inhibits Viral Infection via Translational Suppression

Moon Jung Song, Ph.D., *Korea University College of Life Sciences and Biotechnology, Seoul, South Korea*
Maria Razzaq, *Sungkyunkwan University College of Medicine, South Korea*
Ji Ho Han, *Korea university College of Life Sciences and Biotechnology, Seoul, South Korea*
Subramaniyam Ravichandran, Ph.D., *Sungkyunkwan University College of Medicine, Suwon, South Korea*
Jaehyun Kim, *Korea university College of Life Sciences and Biotechnology, Seoul, South Korea*
Joon-Yong Bae, Ph.D., *Korea University College of Medicine, Seoul, South Korea*
Man-Seong Park, Ph.D., *Korea University College of Medicine, Seoul, South Korea*
Woo-Chang Chung, *Sungkyunkwan University College of Medicine, Suwon, South Korea*
Jin-Hyun Ahn, Ph.D., *Sungkyunkwan University College of Medicine, Suwon, South Korea*
Kyeong Kyu Kim, Ph.D., *Sungkyunkwan University College of Medicine, Suwon, South Korea*

The G-quadruplex (G4) formed in single stranded DNAs or RNAs plays a key role in diverse biological processes and is considered as a potential antiviral target. In the genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), 25 putative G4-forming sequences are predicted; however, the effects of G4-binding ligands on SARS-CoV-2 replication have not been studied in the context of viral infection. In this study, we investigated whether specific G4 ligands suppress SARS-CoV-2 replication and whether their antiviral activity involves stabilization of viral RNA G4s and suppression of viral gene expression. We found that pyridostatin (PDS) suppressed viral gene expression and genome replication as effectively as the RNA polymerase inhibitor remdesivir. Biophysical analyses revealed that the 25 predicted G4s in the SARS-CoV-2 genome form a parallel G4 structure. In particular, G4-644 and G4-3467 located in the 5′ region of ORF1a, formed a G4 structure that could be effectively stabilized by PDS. We also showed that PDS significantly suppressed translation of the reporter genes containing these G4s. Taken together, our results demonstrate that stabilization of RNA G4s by PDS in the SARS-CoV-2 genome inhibits viral infection via bio translational suppression, highlighting the therapeutic potential of G4 ligands in SARS-CoV-2 infection.

808. Exoribonuclease Activity as a Validated Target to Fight Arenaviruses and Coronaviruses Infections

Karine Alvarez, Ph.D., *AFMB UMR 7257, CNRS, AMU, Marseille, France*
Sergio Hernandez, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
Mikael Feracci, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
Carolina Trajano de Jesus, M.S., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
Clémence Mondielli, Ph.D., *Evotec, Toulouse, France*
Fabrice Bailly, Ph.D., *INSERM, CHU Lille, UMR-S 1172, Lille, France*
Philippe Cotelle, Ph.D., *INSERM, CHU Lille, UMR-S 1172, Lille, France*
Franck Touret, Ph.D., *UVE : Aix-Marseille University -IRD 190-Inserm 1207-IHU Méditerranée Infection, Marseille, France*
Xavier De Lamballerie, Ph.D., *UVE : Aix-Marseille University -IRD 190-Inserm 1207-IHU Méditerranée Infection, Marseille, France*
Bruno Coutard, Ph.D., *UVE : Aix-Marseille University -IRD 190-Inserm 1207-IHU Méditerranée Infection, Marseille, France*
Etienne Decroly, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
Bruno Canard, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*
François Ferron, Ph.D., *AFMB UMR 7257 CNRS, AMU, Marseille, France*

Arenaviruses are emerging RNA viruses associated with fatal neurological and hemorrhagic diseases in humans with limited therapeutic options. For coronaviruses, in the context of the global SARS-CoV-2 pandemic, it is essential to develop potent antivirals, in support of the vaccine approach. To date, viral exoribonucleases (ExoN) have only been identified in the Arenaviridae and the Coronaviridae families. The ExoN activity carried by arenavirus nucleoprotein or the nsp14 protein of coronavirus participates to the suppression of the host innate immune response, moreover the nsp14 displays an additional role in maintening the genome integrity. These ExoN, belonging to the same DEDD superfamily, are characterized by a TMIC in the RNA hydrolysis mechanism. Because of their key roles in virus life cycle, they constitute attractive target for drug design. We developed a sensitive, robust and amenable to miniaturization, fluorescence polarization assay to measure the ExoN activity and its inhibition in vitro. The effectiveness of the method was validated on three different viral ExoN, including SARS-CoV-2, Lymphocytic Choriomeningitis and Machupo viruses. We performed a screening of a focused library consisting of 113 metal chelators. We identified several hit inhibitors and determined by fluorescence polarization their IC50 at a micromolar level. Then we validated their activities in cell culture assays and we found 3 compounds displaying efficiency at micromolar level against SARS-CoV-2 infected Vero-E6 cells. These results highlight the effectiveness of fluorescence polarization assay for the discovery of antivirals. doi.org/10.1016/j.antiviral.2022.105364

809V. Antiviral Activity of Catechol and Its Derivatives Against Epstein-Barr Virus

Min Sun Park, B.S., *Korea University, Seoul, South Korea*
Ye-Jin Kim, M.S., *Korea University, South Korea*
Moon-Jung Song, Ph.D., *Korea University, Seoul, South Korea*

Catechol, a phenolic compound found in various fruits, vegetables, and woods, was first discovered in the plant extract catechin. Although catechol and its structural derivatives are shown to exhibit anticancer, anti-inflammatory, antioxidant, and antimicrobial activities, little is known about their ability to inhibit virus replication. Epstein-Barr virus (EBV) is an important human oncogenic virus, as it is associated with various malignancies such as Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, and gastric cancer. In this report, we evaluated the antiviral effects of catechol and its derivatives such as 3-methylcatechol, 4-methylcatechol, 3-methoxycatechol, and 4-ethylcatechol, against EBV. Upon reactivation of EBV, catechol and its derivatives efficiently reduced the viral lytic protein expressions, Zta and EA-D, encoded by BZLF1 and BMRF1, respectively, in several EBV-positive B cell lines as well as in an EBV-associated gastric cell line in a dose-dependent manner. They also reduced the levels of viral transcripts, BZLF1 and BMRF1, and inhibited the transactivation of BZLF1, BMRF1, BMLF1, and BHLF1 promoters induced by ZTA and/or RTA in a dose-dependent fashion. The results from time-of-addition experiments showed that these compounds suppressed viral lytic replication during the early phase of viral reactivation, although it did not affect the activation of the MAPK signaling pathway. Taken together, our results suggest that catechol and its derivatives exert antiviral effects against EBV without inducing cytotoxicity in vitro, thereby serving as potential preventive or therapeutic reagents in EBV-associated diseases.

NOTE: Numbers denoted in bold are abstracts for which the author listed is the presenting author.

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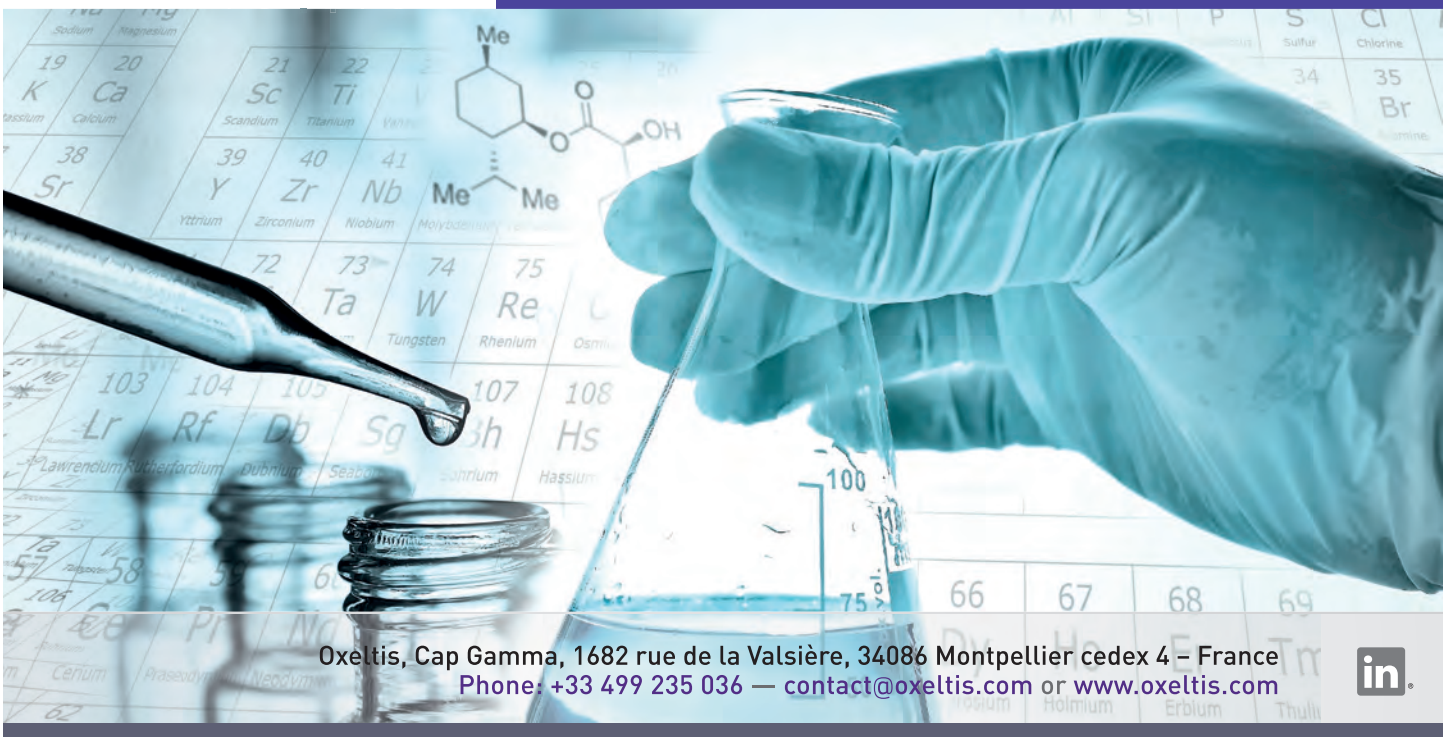

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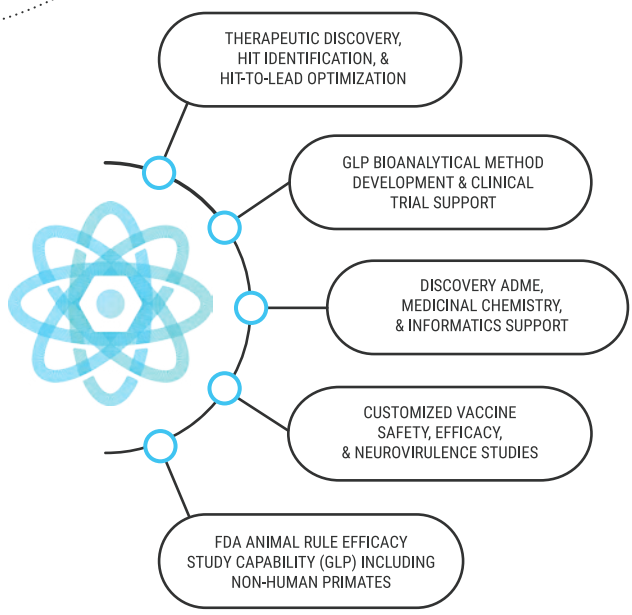

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