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Meeting report: 35th International Conference on Antiviral Research in Seattle, Washington, USA – March 21–25, 2022

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A B S T R A C T

The 35th International Conference on Antiviral Research (ICAR), sponsored by the International Society for Antiviral Research (ISAR), was held in Seattle, Washington, USA, on March 21–25, 2022 and concurrently through an interactive remote meeting platform. This report gives an overview of the conference on behalf of the society. It provides a general review of the meeting and awardees, summarizing the presentations and their main conclusions from the perspective of researchers active in many different areas of antiviral research and development. Through ICAR, leaders in the field of antiviral research were able to showcase their efforts, as participants learned about key advances in the field. The impact of these efforts was exemplified by many presentations on SARS-CoV-2 demonstrating the remarkable response to the ongoing pandemic, as well as future pandemic preparedness, by members of the antiviral research community. As we address ongoing outbreaks and seek to mitigate those in the future, this meeting continues to support outstanding opportunities for the exchange of knowledge and expertise while fostering cross-disciplinary collaborations in therapeutic and vaccine development. The 36th ICAR will be held in Lyon, France, March 13–17, 2023.

1. Introduction

The International Conference on Antiviral Research (ICAR) is the annual meeting of the International Society for Antiviral Research (ISAR). The 35th annual ICAR was held on March 21–25, 2022, using a hybrid model with in-person sessions in Seattle, WA, USA, and concurrent live virtual sessions on the OnAIR virtual event platform. This was the first in-person ICAR since the SARS-CoV-2 pandemic began in late 2019. As in previous years (Andrei et al., 2017; Brancale et al., 2022; Tramontano et al., 2019; Vere Hodge, 2003a, 2003b, 2011, 2013, 2014, 2015) the following report provides an overview of the conference based on meeting notes and the conference abstract book, including the 2022 (and one 2020) awardee lectures, synopses of the session lectures, and summaries of the results of the Chu Family Foundation awards as well as the poster and PechaKucha competitions.

ICAR 2022 began with the opening session and plenary speakers followed by ICAR awardee lectures interspersed between speaker sessions. The six speaker sessions focused on non-coronavirus respiratory viruses, retroviruses and other viruses, broad spectrum antiviral drugs and pandemic preparedness, arboviruses, coronaviruses, and hepatitis and herpes viruses. These sessions featured speakers from around the world, including Asia, North America, South America, Europe, and Australia. Special sessions and events included the Women in Science Roundtable, PechaKucha competition, late-breaking oral presentations, a career development interactive workshop hosted by Prof. Harmit Malik of the Fred Hutchinson Cancer Research Center, Seattle, WA, and a closing dinner event. The poster sessions were held both in person and on the virtual platform, complementing the sessions and presenting additional advances in all areas of antiviral research and development. This year's ICAR was especially timely given the critical role of many researchers in various fields represented at the meeting who are responding to the ongoing SARS-CoV-2 pandemic. ICAR specifically highlighted the key role of research on both the virological and chemical aspects of antiviral drug discovery and the developments in

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academia, industry, health care, and other national, international, governmental, or not-for-profit organizations in combating viral threats. Altogether, the sessions, short talks, shotgun talks, and poster presentations once again gave an excellent background and update on the broad field of antiviral research and continued challenges posed by viral pathogens.

2. ISAR awards

2.1. 2022 Gertrude Elion memorial award. Developing an Oral Antiviral Agent in a pandemic: The Evolution of Molnupiravir (EIDD-2801, MK-4482) as a treatment for COVID-19. George Painter, Ph.D., EIDD, DRIVE, Department of Pharmacology and Chemical Biology, Atlanta, Georgia, USA

The Gertrude Elion Award is given annually to an outstanding scientist, not necessarily in the field of antiviral research but certainly someone who has made considerable contributions to the scientific field directly or peripheral to it. It was particularly apt that **Prof. George Painter** received this award as its namesake, Dr. Elion, hired George into his first industry position. During the opening session, George presented his award lecture entitled "Developing an Oral Antiviral Agent in a Pandemic: The Evolution of Molnupiravir (EIDD-2801, MK-4482) as a Treatment for COVID-19". Molnupiravir was developed at DRIVE (Drug Innovation Ventures at Emory) where work had begun in partnership with the Defense Threat Reduction Agency (DTRA) to identify a drug candidate for VEEV in one year. The target product profile called for a broad-spectrum, oral agent that could be rapidly deployed to soldiers in the field or to the public in a health emergency. Recognizing that most antiviral drugs traditionally have been nucleotide or nucleoside inhibitors, either as stand-alone agents or in combination with other molecules, and that RNA-dependent RNA polymerases (RdRPs) have been excellent targets for antiviral drug discovery over the years, it wasn't surprising that this class of molecules was pursued. Given the high degree of conserved structures for many RdRPs, it was hoped that this approach would also lead to broad spectrum antiviral agents.

One significant challenge to the development of a VEEV therapeutic was the need to adhere to the animal rule for clinical development. This rule posed a challenge in that the original lead molecule, EIDD-1931, had a significant decrease in oral bioavailability in non-human primates (NHPs), which was the most relevant species for use under animal rule. Investigation into the mechanism(s) leading to this low bioavailability, which was unique to NHPs as a species, determined that the molecules were being trapped in the gut due to triphosphorylation in enterocytes. A prodrug of EIDD-1931 was developed, designated as EIDD-2801 (Molnupiravir), which was active by IV administration, thus bypassing the gut trapping, and so became the clinical candidate.

Unlike many nucleoside analogs, EIDD-2801 acts as a competitive alternative substrate for the RdRP, not an inhibitor. Moreover, the nucleobase can pair with A or G. Following incorporation into the replicating viral genome, the hydroxylamine can tautomerize to its imino form leading to mismatches. At a certain point, the number of mutations introduced to the viral genome through these base mispairings passes a tolerable threshold, leading to viral error catastrophe with significant impairment of complete loss of viral replication. Data has demonstrated that not many mutations are needed to achieve this antiviral effect.

Given its mechanism, an extensive program was necessary to demonstrate that the molecule would not be mutagenic to humans, which would require a 2′-deoxy conversion of the compound. After a lengthy investigation, the regulators ultimately agreed that the weight of evidence demonstrated a low risk of mutagenicity.

In addition to activity against VEEV, EIDD-2801 also demonstrated potent activity against pandemic influenza, chikungunya virus, and pathogenic coronaviruses. Shortly after the SARS-CoV-2 virus emerged, EIDD-2801 was also shown to be potent against this virus and demonstrated a high barrier to resistance. In March 2020, DTRA reached out to DRIVE to proceed with an IND for EIDD-2801 against COVID-19. The clinical program moved rapidly through phase 1 single and multiple ascending dose studies. For phase 2, infectivity was the key virology endpoint with viral cultures at baseline, day 3, day 5. Following phase 3 trials, Molnupiravir was approved in the UK in November 2021 and shortly thereafter received an EUA in the US and other countries.

2.2. 2020 Antonín Holý memorial award. Fleximers – a strategic approach to broad-spectrum antiviral therapeutics. Kathie Seley-Radtke, Ph.D., University of Maryland, Baltimore County, USA

Prof. Kathie Seley-Radtke finally got the opportunity to give her 2020 Holy award lecture entitled "Fleximers – a strategic approach to broad-spectrum antiviral therapeutics" after a delay of two years due to the pandemic. After a hilarious introduction including numerous photos by Dr. Chris Meier, also a former Holy award winner, that revealed several important aspects of her personal life such as her love of wine, more wine, dogs and cooking, Kathie gave us a nice summary of not only the historical development, but also the current results, for her unique nucleoside scaffold known as fleximers. The fleximers feature a "split" purine ring system that is connected by a single carbon bond between the resulting two heterocyclic pieces, typically an imidazole and a pyrimidine. This strategic feature has been shown to endow the nucleoside scaffold with several key advantages. Because of the additional flexibility, the fleximers can rotate and/or reposition to avoid point mutations, as well as to increase binding affinity by engaging secondary amino acid residues in the target enzyme binding site that weren't previously involved in the mechanism of action. In addition, this has been shown to result in activity against viruses that the parent nucleoside has no activity against. One example is the flex-acyclovir series, which have shown low single digit antiviral activity against Ebola, Marburg, SARS-CoV-1 and MERS, while acyclovir exhibits no activity. Similarly, several of the fleximers have shown low micromolar to nanomolar levels of activity against flaviviruses such as dengue, zika, yellow fever and tickborne encephalitis, while again, acyclovir exhibits no activity against those viruses. Recent studies in mice have shown that several of the fleximers including a new series related to AT-511/527, are safe and well tolerated by mice, even in 10-day studies at high concentrations. She wrapped up her talk by showing the audience some of the newer scaffolds her group are pursuing, including several related to drugs being developed for treatment of SARS-CoV-2 and other viruses of pandemic concern.

Overall, it was an excellent summary of many years of work, encompassing all aspects of a collaborative medicinal chemistry project including organic synthesis, computational modeling, antiviral testing *in vitro* and *in vivo*, as well as biochemical mechanism of action studies. Kathie then highlighted how attending ICAR since 1991 has led to her numerous collaborations and strong friendships, showing a slide filled with many familiar faces, several whom were in the audience or attending virtually.

2.3. 2022 Antonín Holý memorial award. Structure-guided antiviral drug discovery – a tale of two viruses. Mark von Itzstein, Ph.D., Institute for Glycomics, Griffith University, Gold Coast, Queensland, Australia

The Antonín Holý memorial Award is bestowed annually to a medicinal chemist who has made major contributions to antiviral research. This year's awardee was **Prof. Mark von Itzstein**, Griffith University, Australia, who delivered the lecture at 8:30 a.m. – half past midnight Australia time! Mark thanked the ISAR Awards Committee and expressed how humbling it was for him to see so many past Holý Awardees in the audience.

Mark highlighted the importance of emerging infectious diseases, stressing the importance of influenza, with a high pandemic risk, evolution through reassortment, and limited cross-protection conferred by current vaccines, and human parainfluenza virus (HPIV), which has a significant health impact in the elderly and the young and for which no vaccines or antivirals are approved.

Respiratory human viruses, including influenza, HPIV, and SARS-CoV-2, use carbohydrate receptors. Thus, understanding the "language" of the virus-carbohydrate interactions helps in developing antivirals. The classical model proposed one virus interacting with one receptor, which Mark illustrated with the metaphor of couples waltzing, in which each person interacts with only one partner. However, updated models consider that viruses behave more like square dancers in this analogy, where each person interacts with many different partners. Mark related that his early academic interest in influenza neuraminidase evolved into the application to therapeutics.

Replication of influenza virus starts by binding to glycan receptors by the viral hemagglutinin (HA - a lectin) and ends when an exoglycohydrolase (neuraminidase - NA) cleaves a terminal α-ketosidically linked Neu5Ac, thus releasing the replicated virions. The NA inhibitors (zanamivir and oseltamivir) thus prevent this release, and the budded virions clumped on the surface of the infected cell provide a good target for the immune system. Mark briefly discussed other types of influenza drugs. Mark's main focus is on structure directed drug design. These studies have shown the tight binding of sialic acid to NA, with one carboxylic acid driving the engagement. Modeling suggested that a sulfonate moiety in the sialic acid equatorial position would lead to potent and specific inhibitors. As no sulfonated NA inhibitors had been described, Mark's group developed the chemistry to synthesize these derivatives. Equatorial position of the sulfonate group produced compounds with potency similar to that of phosphonate analogs. Mark described the chemistry for the introduction of a sulfonate group into zanamivir (sulfozanamivir). The approach produces both the axial and equatorial derivates, whose separation is straightforward. The equatorial sulfonated sulfozanamivir is as potent as zanamivir in cell-based assays, and the binding mode was confirmed by structural biology. Mark's group are now focused on producing long-acting drugs that would require only one treatment.

Mark introduced his group's current focus on fragment-based drug discovery based on saturation transfer difference nuclear magnetic resonance (STD NMR) ^{19}F spectroscopy. The typically weak fragment binding is optimized through synthetic medicinal chemistry iterations. A library of >500 fragments were screened against bacterial and viral NA. Binding fragments were verified and advanced into structural biology studies to identify binding modes. The fragments identified have no commonalities with known drugs and may be broad spectrum NA inhibitors. Mark was very excited about this approach.

Mark also discussed that a single HPIV protein, HN, has HA and NA activities. HN has a single pocket that both binds and releases sialic acid. NA activity is activated at low pH (4.6–5.0), whereas binding occurs at pH 7.0. A flexible "216-loop" allows the binding site to fit bulky C-4 substituents. Mark's group has used structural biology guided chemistry to improve on existing weak NA inhibitors with no bulky substituents, increasing their potency from the milli-to the micro-molar ranges. The bulky moieties push the 216 loop further back to nicely fit into the larger binding pocket. Newer compounds are reaching potencies in the nanomolar range.

During the question period, Mark described that the Neu5Ac2en highly potent NA inhibitors, which have little HA inhibitory activity, fail to inhibit viral replication. Mark thinks that both HA and NA functions must be inhibited. Mark also indicated that they have screened many existing drugs but have found no inhibitors of the NA binding site among them.

In summary, Mark delivered an outstanding talk describing his journey from an academic interest in using structural biology and chemistry to study neuraminidases to the development of clinical antivirals and the further exploration of NA inhibitors as antivirals against important human respiratory viruses.

2.4. 2022 William Prusoff memorial award. Targeted Protein Degradation as an Antiviral Strategy. Priscilla Yang. Ph.D., Stanford University School of Medicine, Stanford, California, USA

The William Prusoff Memorial Award is given annually to an outstanding young scientist who has demonstrated dedication and excellence in the field of antiviral research (basic or clinical, synthetic or pharmacological) as well as future potential for contribution to the field and the society. Accepting the 2022 award, **Prof. Priscilla Yang** presented a talk entitled "Targeted Protein Degradation as an Antiviral Strategy".

Priscilla approaches antiviral drug discovery by searching for opportunities to tackle gaps in current approaches. She notes that most direct acting antivirals (DAAs) have a narrow spectrum of activity. Additionally, resistance often arises rapidly, especially for RNA viruses. Functional genomics or genetic screens for antiviral targets have identified several good viral candidates that don't appear druggable due to the lack of known enzymatic activities or information regarding the protein's biochemical activity.

Targeted protein degradation (TPD) provides an alternative approach to conventional enzymatic inhibition. The discovery of the TPD approach emerged from the mechanism of Thalidomide which brings the E3 ligase complex cereblon together with neosubstrates to facilitate their degradation through the ubiquitin degradation pathway. Due to this mechanism, a compound that facilitates TPD of a viral protein only needs to bind the protein, not inhibit any of its functions. Rather, by targeting the protein for degradation, all functions of that viral protein are inhibited, including both those that have been identified as well as those that have not.

As opposed to traditional small molecule enzymatic inhibitors, which elicit their effects by occupancy driven inhibition, targeted protein degraders function through event driven pharmacology meaning

that a transient binding event is sufficient for activity, and thus a weak binder can still be effective.

There were several potential challenges or disadvantages to the TPD approach proposed by Priscilla. First, many viral proteins locate in specialized compartments that may not be accessible to the E3 ligases that the degraders are designed to target. Second, many viral proteins are quite abundantly produced in cells and could overwhelm the degradation machinery. Finally, there may be cytotoxicity due to the degradation of these proteins. Exploration of different types of degraders designed for various viral proteins may help elucidate which of these potential issues may arise.

Priscilla presented data from her lab demonstrating that antiviral targeted protein degraders could be made by fusing a known HCV NS3 binder, telaprevir, with the cereblon binding domain of a thalidomide-like molecule. These fusion molecules demonstrated both antiviral activity as well as direct degradation of NS3. Several studies were undertaken to demonstrate that the fusion proteins were degrading NS3 through a cereblon-dependent mechanism and were specifically degrading NS3 rather than inducing non-specific degradation. Further studies showed that these fusion molecules had advantages compared to the parental telaprevir alone in terms of breadth of activity and overcoming resistance to telaprevir. Additionally, serial passage with the degraders did not yield any resistant virus.

A second program creating degraders from GNF-2, a binder of Dengue virus (DENV) E protein that is not an assembly inhibitor, was undertaken. The DENV E protein is a particularly interesting protein to target given its location in the endoplasmic reticulum (ER). The GNF-2 based degraders were able to degrade E and to do so in a cereblon-dependent manner. GNF-2 is also known to inhibit Abl kinase. To separate the E degrader activity from the Abl kinase inhibition, an independent compound was generated that retained E binding but lost Abl binding. This compound retained potent antiviral activity. Mechanistic studies demonstrated that this particular degrader did affect entry, but that the effect was not due to degradation. Moreover, assembly inhibition of the compound appeared to be dependent on its degradation activity. To explore the breadth of activity for this molecule, it was assessed for antiviral effect against Zika, JEV, and WNV and found to be active against all of them.

This work provides proof of concept that TPD is a viable platform for antiviral drug discovery, providing access to a broad range of viral targets that had not previously been druggable.

2.5. 2022 Women in Science Award. Fighting viral hemorrhagic fevers: from the benches to the trenches. Christina Spiropoulou, Ph.D., Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Dr. Christina Spiropoulou was presented with the ISAR Women in Science Award, which is given annually, for her achievements as a senior scientist in the Viral Special Pathogens Branch at the CDC. Her title, "Fighting viral hemorrhagic fevers: from the benches to the trenches", reflects her work in the laboratory, particularly the BSL-4 facilities, and field responses to outbreaks and eco-investigations.

The first part of Christina's presentation described the "benches" in BSL-4 at the VSPB where studies are conducted using molecular techniques and animal models to develop vaccines and antiviral drugs for hemorrhagic fever viruses in the Filo-, Arena-, Bunya-, Paramyxo-, and Flaviviridae. They use a universal reverse genetics approach to generate recombinant viruses with a ZsGreen1 (ZsG) reporter gene fused to the N or M gene with a P2A linker separating the ORFs. Live recombinant reporter viruses were constructed from sequence data for EBOV Ituri, and Bombali (BOMB), a newly discovered filovirus in bats in West Africa. These ZsG viruses are invaluable for studying pathogenesis and immune responses in cells and animal models, including mouse, humanized mouse, Syrian hamster, guinea pig, and ferret. Important considerations are 1) does the reporter attenuate the virus; 2) is the virus virulent *in vivo*; and 3) is the disease comparable to WT virus? For example, Crimean Congo Hemorrhagic fever (CCHF) virus with ZsG is comparable to WT CCHF virus *in vitro* and in an IFNAR$^{-/-}$ mouse model. Remarkably, the green fluorescence is visible in hamster brains, lungs, and nose infected with Nipah ZsG virus. The ZsG viruses are also useful for high-throughput screening in BSL-4 in 384-well plate formats. She described screening an FDA library and encouraged people to send her their chemical libraries to test.

Christina then highlighted some successes in vaccine development from her group and collaborators. She noted that it was easy to construct a live attenuated vaccine for Rift Valley fever virus with the NSm and NSs virulence factors deleted. Denoted as DDVAX, it is a veterinary vaccine that is safe in rodents, non-human primates, sheep, cattle, and goats. DDVAX is being developed for humans in a project with the Coalition for Epidemic Preparedness Innovations (CEPI), a non-profit foundation. For Lassa fever virus, a VRP (virus replicating particle) approach was taken because it has been shown to be safe, efficacious, and rapidly adaptable. VRPs are morphologically identical to the virus but lack a critical component. The structural glycoproteins were removed from the LASV genome, and the gp genes were then expressed in a cell line. VLPs made in the complementing cells will infect normal cells but not spread. A single dose given 28 days pre challenge can protect guinea pigs against challenge with homologous or heterologous viral strains, and protected 100% of cynomolgus macaques. A single dose can also protect against lethal outcome when given 1 day post challenge. The VRPs for CCHF and Nipah viruses are currently in development and to date, look promising as single-dose vaccines.

In addition, the ZsG recombinant viruses were useful for studying remdesivir and other antiviral strategies. Christina showed that an F548S mutation in the EBOV L polymerase confers 4-fold resistance only to remdesivir and no other drugs, shedding light on the drug's unique mechanism of action. In addition, remdesivir protected African green monkeys from a Nipah virus challenge. A new remdesivir prodrug with a lipid monophosphate, ODBG-P-RVn, was active in cultured cells against EBOV, SARS-CoV-2, yellow fever virus, and Nipah virus. Notably, ODBG-P-RVn is orally bioavailable, so *in vivo* studies are planned. Defective interfering (DI) particles were effective therapeutics for Nipah virus. EIDD-2749 (4′-fluorouridine) was very active in cells against Lassa, Junin, Lujo, and Machupo viruses, thus it will be tested in guinea pigs. However, she made a strong point that there remain very few therapeutic options to fight CCHF, Lassa, or Nipah. Clinicians' top choice is ribavirin, but it does little to help.

The second part of her presentation described the "trenches" of field research during VHF outbreaks. Notably, Christina was on the team to bring diagnostics to Uganda during the EBOV outbreak in 2000, where nested RT-PCR was used for the first time. Supportive care was the only treatment available for people who tested positive for EBOV. In the 2012 EBOV outbreak, she went to the Democratic Republic of the Congo (DRC) and used qRT-PCR and TaqMan to try to improve the diagnostics. Although the diagnostics were better, available treatments remained poor. In 2014–2016, during the large outbreak in West Africa, diagnostics were good, and several therapeutics were tested including convalescent plasma, favipiravir, ZMapp, remdesivir, among others. Attention to EBOV was high after this outbreak, and a strong effort to develop antivirals and vaccines appeared to be underway. When EBOV

broke out again in 2018 in the DRC, the PALM clinical trial for therapeutics was quickly started. The MAB114 and the REGN-EB3 cocktails proved better than ZMapp or remdesivir, and both were licensed by the FDA in 2020. In 2021, outbreaks in the DRC and Guinea were genetically linked to earlier outbreaks in those countries – raising the concern that the outbreaks arose from persistent viral infection. This theory has been confirmed, and it is now known that EBOV persists in immune-privileged sites (semen, eye, CNS, uterus). Related to this, Christina then stressed that we need to consider treating the people who survive EBOV infection to eradicate persistent virus.

Christina concluded that more research needs to be done for conquering viral hemorrhagic fever diseases. She sees a need for new and better monoclonal antibodies, small molecules that can enter immune privileged sites, and for therapies that cross the blood-brain barrier. She urged that we need more research to understand viral persistence and recrudescence, especially for EBOV, and how therapeutics can hopefully address this problem. Indeed, during the question-and-answer period, Dr. Priscilla Yang asked whether mAbs have been combined with remdesivir and Christina replied that yes, this was a good idea and should be tested.

2.6. 2022 Diversity Award. The COVID-19 pandemic: a view from the bench. J. Victor Garcia-Martinez, Ph.D., University of North Carolina, Chapel Hill, North Carolina, USA

Prof. Victor Garcia-Martinez gave a fascinating presentation highlighting his group's critical work on HIV and the subsequent pivot to develop an innovative mouse model to support key SARS-CoV-2 antiviral screening studies of EIDD-2801 (Molnupiravir). He began by discussing his work on HIV and noted that while great progress has been made in developing antivirals against this virus, all these antivirals depend on continued treatment, and when treatment ceases, the virus reactivates. This challenge is due to HIV's integration into the genome of resting $CD4^+$ T cells. Victor's lab sought to test agents that reverse this latency, evaluating many compounds with this mechanism of action. While most compounds worked *in vitro*, the group met with little success when trying to repeat this effect *in vivo*. The turning point was a new molecule, designated as AZD5582. Victor's group used BLT mice, which are humanized mice systematically reconstituted with human immune cells, conferring susceptibility to infection, to test AZD5582 and observed latency reversal in resting $CD4^+$ cells in almost all tissues, except the spleen. This work was published in *Nature*. A week later, the group was faced with the coronavirus pandemic, which had, by then, reached global attention. Like many other research groups, Victor's lab was "relegated to the back bench" – the lab was to be closed and the lab members told to go home.

This prompted a new focus within the group based on the desire to advance model availability for SARS-CoV-2 and many other human pathogens. As a result, the group developed a human lung-only mouse (LoM), which Victor showed can support *in vivo* replication of many clinically relevant human pathogens, permitting the evaluation of diverse antiviral approaches. LoM mice are generated by human lung tissue engraftment, providing ectopic lungs (not used for breathing) with tissue that expands, grows, and expresses 40 different types of human cells. Viruses (like CMV) that do not infect mice are able to replicate in the engrafted human tissue in LoM mice. Further work evaluated a wide variety of pathogens: *Mycobacterium tuberculosis*, influenza virus, respiratory syncytial virus (RSV), cytomegalovirus (CMV), and Middle East respiratory syndrome-related coronavirus (MERS-CoV). Using the LoM model, the group looked at human cell types susceptible to infection and evaluated activity of antiviral compounds. They investigated several human and bat-borne coronaviruses and confirmed ACE2 expression and viral replication in tissue, demonstrating virus infection in LoM without the need for virus adaptation. This result allowed the LoM model to serve as a single platform for directly comparing viruses and therapeutic interventions. The group performed detailed sequence analyses looking at immune gene regulation to gain insight into pathogenesis of SARS-CoV-2. They then used the model to demonstrate both prophylactic and post-exposure efficacy of EIDD-2801 against SARS-CoV-2 infection, and these results were published in *Nature*.

Finally, Victor discussed how LoM animals closely mimic SARS-CoV-2 human pathology resembling lung fibrosis in engrafted tissue; SARS-CoV-2 infection induced markers of fibrosis (collagen A1 and smooth muscle actin), which were detected even a month after infection. Using the model, his group was able to address questions of long-term sequelae and the role that antiviral treatment can play in mitigating clinical consequences of viral damage to pulmonary tissues. Importantly, the group showed that EIDD-2801 reduced markers of pulmonary fibrosis, supporting use of early antiviral treatment to prevent long-term sequelae.

3. ISAR plenary speakers

3.1. What role can foundations play in driving innovation globally in antiviral development? Ken Duncan, Ph.D., Bill & Melinda Gates Foundation, Seattle, Washington, USA

Dr. Ken Duncan's talk focused on the core values of the Gates Foundation as well as several new initiatives in which they are involved. In that regard, the Gates Foundation believes strongly in the tenet that "all lives have equal value" and that every person is entitled to a healthy and productive life. These basic beliefs are what underpins the Foundation's mission. Their goal is to have a transformational impact and provide tools to help raise the disadvantaged out of poverty. To achieve this goal, the Gates Foundation partners with many different organizations, including various private foundations, government agencies, industry, and other entities.

The Foundation is primarily focused on accelerating antiviral research space while creating global equity. The hope is also to inspire others to change policies, attitudes, and our ways of thinking about how we can increase access to drugs for low-income countries. It is critical that we develop drugs and vaccines that are suitable for use and distribution globally, particularly in low-income and low-resource countries. This approach includes developing small molecules that can easily be stockpiled and distributed, as well as developing diagnostics that can be used in the field. This goal can be accomplished by engaging scientists globally, bringing programs together and sharing data, and establishing funding partnerships with many private and public players.

While we continue to make progress on pan-active coronavirus agents, there is still room for innovation. For example, the high pill burden for the two EUA oral COVID drugs, the drug-drug interactions with ritonavir, as well as the potential for resistance with using single agents, pose some limitations. Combination therapies are clearly going to be important. In addition, pills that would give several days of protection would be of great value. Dr. Duncan then discussed the lessons learned from the pandemic – just how unprepared we were, the lack of investment in the development of CoV antivirals after the SARS and MERS outbreaks, the limited impact repurposing had on the pandemic, and the need to develop prophylactic agents. It is critical that we must be prepared to address the viral families that have the most potential for the next pandemic.

In that regard, the Foundation is now involved in a new initiative – the Pandemic Antiviral Discovery (PAD) program which arose through a group of philanthropic groups including the Novo Nordisk Foundation and Open Philanthropy. The goals for the target product profile (TPP) are global equity, oral administration, no monitoring requirement, affordability, scalability, stability, no cold chain requirement, suitability for drug combinations, no drug-drug interactions, and a global regulatory pathway. New drug candidates discovered through this initiative should ideally be Phase II ready. The first call will focus on henipaviruses, but there will be additional calls soon.

In summary, Ken believes that we need to be able to respond quickly to a new threat with rapid decision making and grant awards. We need to pursue innovation suitable for global use by taking risks, and using a diverse approach not limited by the traditional guidelines that have slowed drug development. Moreover, we need a global call to action to build capacity, strengthen institutions, and make drugs and vaccines rapidly available. To reach these goals, it is critical to establish partnerships between government funding agencies, academicians, nonprofits, industry, and philanthropic entities. While it will be difficult to coordinate multiple players to avoid overlap, duplication of efforts, and differing agendas, it can be accomplished if we have a common goal and work together.

3.2. Drug development for viruses of pandemic potential, where are we now and where are we going? Carl W. Dieffenbach, Ph.D., National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA

Dr. Carl Dieffenbach began his talk with a bang, showing a photo of the program from the fifth ICAR conference from 30 years ago where he was a speaker. At the time he had been working on coronaviruses (CoVs), and others kept on asking him why he was wasting his time on those viruses! He then switched to working on HIV, and his entire career was spent focusing on HIV until the pandemic. Carl then went on to discuss how many of the lessons learned from HIV can teach us much as we now continue to target CoVs.

Carl outlined the history of HIV/AIDS, covering some of the early (and sometimes crazy) hypotheses, and some of the major advances. He noted that interestingly, HIV was initially diagnosed based on symptoms, much like CoVID is today. He then outlined the importance of accurate measurement, and how three assays were developed that helped us gain a better understanding of the disease and how to treat it. One of those assays was the antibody test, which proved invaluable, and led to the development of protease inhibitors and nucleoside inhibitors to be the first drugs to treat HIV. Not coincidentally, these are the same classes of drugs first approved to treat CoVs. The second assay was the PCR method, which helped researchers study the effectiveness of antivirals in people by monitoring viral load. The third assay looked at the CD4 count. As Carl noted, Dr. John Mellors (University of Pittsburgh) described the viral load would be the speed of the train, while the train position on the track was the CD4 count – a concept that still holds true today.

Another lesson learned involved the evolution from monotherapy to combination therapy, as resistance quickly emerged to the early HIV drugs used as monotherapies, necessitating a new approach which led to the combination of three agents that resulted in a profound drop in viral load. Today, we have numerous combination drugs available for treatment, and we not only know what to start treatment with to achieve best effect, but also when and where they can be used, including for prophylaxis. The next chapters for HIV treatment and prevention will focus on long-acting drugs, PREP and hopefully, someday, vaccines and even a cure, both of which have been elusive.

Carl then segued into the current pandemic and noted that we were amazingly successful in compressing years of work into a two-year time frame to fight COVID. Regardless, as he stressed, we need to be better prepared for the next outbreak. In that regard, NIH is investing $3.2B to develop new direct acting antivirals CoV-2 as well as pandemic preparedness measures for other viruses. Carl also noted that the origin of CoV-2 was indeed the wet market. We also now know that the mode of transmission is through the air, and that the infectivity seems to be increasing with each new variant. Moreover, while vaccines are critical, just as importantly, drugs can have a profound effect on preventing death.

The challenges ahead include the narrow treatment window, which has led to the new "Test2Treat" program that establishes a treatment cascade that will allow a person to be tested at places like Minute Clinics to see a physician, and immediately be prescribed drugs on site. Another challenge is the need for new drugs and more importantly, combination therapies. In that regard, Carl introduced the Antiviral Drug Discovery Centers (AViDD) for Pathogens of Pandemic Concern, which focus on a two-pronged approach of discovery and development by creating research centers to develop inhibitors for seven RNA viral families over a five-year period. Those include CoVs, Bunya-, Filo-, Flavi-, Paramyxo-, Picorna- and Togaviruses. The program will involve all aspects of drug development to create a robust pipeline of antivirals including design, synthesis, structural biology, biochemistry of the viruses, and virology, and produce compounds that can be taken through Phase I, and ready for Phase II, then move to IND with industrial and private/public partners. In addition, government agencies, including NIAID, NCATS and BARDA will be involved. NIH will also offer preclinical service contracts and scale up synthesis with NCATS – basically a "one stop shop" for drug development. Finally, Carl stressed the bottom line – we need to be ahead of the curve next time. We need to be able to respond quickly as well as possibly predict the next outbreak. Moving forward, we must develop new platforms and new drugs by working as a community and harnessing the significant expertise available.

4. Session 1: non-coronavirus respiratory viruses

4.1. Inhibiting entry of human paramyxoviruses. Anne Moscona, M.D., Columbia University Vagelos College of Physicians and Surgeons, New York, New York, USA

Dr. Anne Moscona (Columbia University New York, NY, USA) starting by briefly summarizing the importance of paramyxoviruses in human health. About one fifth of all mortality in children younger than 5 years old result from respiratory infections, mostly with parainfluenza virus, RSV, influenza virus or human metapneumovirus. Entry of paramyxoviruses including parainfluenza virus, as that of other viruses, starts by binding to cell surface sialylated glycans, followed by activation of the viral fusion proteins leading to the fusion between the viral envelope and cellular membrane proceeding through a hemi-fusion stalk. For parainfluenza virus, binding to the sialylated glycans is mediated by the receptor binding protein HN (hemagglutinin -neuraminidase), whereas fusion itself is mediated by F. Together, HN and F form a fusion complex that must be tightly regulated so that the fusogenic conformation is not triggered prematurely. In the mature virion, the globular heads of HN protrude forming an outer layer of the virion, whereas the pre-activation F is buried underneath, at the same depth as the HN stalks. Nonetheless, F interacts with the globular heads of HN, and these interactions are essential to regulate fusion. One of the globular heads of HN is in the up position, to interact with the receptor, and the other in the down position, to interact with F. Mutations in the dimer interface surface of the globular HN head, called site II, result in superfusogenic phenotypes in cell fusion assays, and viruses harboring these mutations are "superfusers" in cultured cells. However, these mutants are not infective to airway epithelium cells in culture or in cotton rat, unless compensatory mutations arise in F or HN II site. The phenotype of these mutants is also consistent with that of the clinical isolates, which are far less fusogenic than laboratory strains, indicating that modulation of fusion is critical *in vivo*. Computational modeling was used to design small molecules that bind to HN site II. The selected

compounds were synthesized, and the active ones were shown to induce conformational change in F and inhibit viral replication in human airway epithelium cells and cotton rats, with titers in airway epithelium cells decreasing by approximately two logs and in cotton rat by one. Cryo-EM shows that in the presence of these compounds, F becomes extended and intercalates in the HN globular head top layer, showing the conformational change that would normally be triggered upon HN binding to sialylated glycans. These results show that the HN interaction prevents the premature F activation until the time when fusion needs to be triggered. Molecular dynamics and structural biology indicated that a neutralizing antibody binds to F at the same site at which F interacts with HN, suggesting that this site in F may also be a target site for antiviral intervention.

Another approach to target F is via peptide inhibitors that disrupt the interaction between the two heptad repeats (HR), HRN and HRC, whose interaction is essential to proceed with fusion. Lipidated versions of these peptides insert themselves in the target cell membrane and inhibit F mediated fusion. These peptides inhibit the infectivity of Nipah virus in hamsters, parainfluenza virus 3 in cotton rats, measles in NHP (and rodents), IAV in cotton rats, Ebola virus in mice and SARS CoV-2 in hamsters and ferrets, and in culture also inhibit that of RSV and human metapneumovirus. Non-natural amino acids were explored to extend the half-life of the inhibitory peptides. The modified peptide had similar potency *in vitro* as noted for the natural ones but exhibited greatly enhanced resistance to proteases. Consequently, they are also more potent in animal models, although not in culture. In conclusion, Anne's talk highlighted the potential of the different entry steps to provide targets for antiviral intervention. During the question-and-answer period, the possibility that these peptides may inhibit virion release was discussed, as it is unknown if the neutralizing antibody targeting F triggers its conformational change or stabilizes it in pre-fusion conformation.

*4.2. Inducing phase transition in viral condensates. A novel Antiviral Strategy. Ralf Altmeyer, Ph.D., Medusa Therapeutics, Hong Kong, Hong Kong SAR, China**

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

4.3. Treatment of EV-D68 respiratory and neurological disease in AG129 mice with a monoclonal antibody, EV68-228. Brett Hurst, Ph.D., Utah State University, Logan, Utah, USA

Dr. Brett Hurst presented the testing of a monoclonal antibody against enterovirusD68 (EV-D68). He started the talk by summarizing the importance of picornaviruses in human health. Besides the well-known poliovirus, Coxsackie A and B viruses and the echoviruses, several of the numbered enteroviruses have been shown to cause significant diseases. They produce mostly respiratory infections, but also flaccid paralysis. EV-D68 was discovered in 1962, but little was known, and while a small number of human cases had been identified, it wasn't until an outbreak of 1395 confirmed cases of EV-D68 were identified in 2014 that more attention was given to this virus. Since then, there have been biannual outbreaks, with numbers decreasing to 30 in 2020. Treatment of these cases is symptomatic, using corticosteroids for inflammation and albuterol for bronchodilation. Brett's group has established two mice models, one for respiratory and one for neurological disease. They both use AG129 mice, which are deficient in the alpha-, beta-, and gamma-IFN receptors. To establish the respiratory model, the virus was passaged serially 30 times in mice lungs. Intranasal infection of four-week-old AG129 infected with $10^{4.5}$ PFU results in high lung titres and viremia ($10^{6,5}$ and, 10^{4}/ml, respectively), as well as high levels of inflammatory cytokines, including IL6 and MCP-1. For the neurological model, 10-day old AG129 mice are infected ip with $10^{6.5}$ PFU, resulting in 100% lethality in 5–6 days with flaccid paralysis, as shown in a video. The neurological disease is then scored in four categories.

The study design included the specific Mab (EV68-228) developed by Dr. James Crowe at Vanderbilt University, a control RSV-specific mAb, and human hyperimmune globulin from convalescent patients. Most groups were treated with 10 mg/kg, but one group received a lower dose of 1 mg/kg, starting from 4 till 24 h post infection. Animals were euthanized at days 3 and 5 to evaluate lung titers. Whereas the single low mAb dose was not effective, and the human hyperimmune globulin had little effect, the higher mAb dose resulted in lower lung titers on days 3 and 5. All treatments were effective at lowering viremia on day 3 (animals are no longer viremic on day 5). Likewise, IL-6 levels were reduced to basal levels by all treatments on day 3 and returned to baseline on day 5. MCP1 levels decreased, too, but in this case the late treatment or lower dose were not as effective.

In the neurological model, treatment with mAb started at either 4 h or 24 h after infection protected against lethality and neurological clinical symptomatology. Delaying the start of treatment till 72 or 120 h resulted in lower protection, but the disease is very advanced at these times in this model. In fact, very few animals were still alive by the time treatment was started. Most treatments were effective at the earlier times, but at the later stages only the specific mAb proved efficacious. During the question-and-answer period, Brett stated that they have not yet evaluated combination treatment with anti-inflammatories and or at the half-life of human mAb in mice. However, they have evaluated the half-life in hamsters, and it was reported to be very long. In summary, Brett's talk described two very interesting models of EV-D68 pathology while showing the potential therapeutic use of EV68-288.

4.4. Impact of PA E23 G/K substitutions on influenza a virus fitness and baloxavir susceptibility, Jeremy Jones, Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee, USA

Dr. Jeremy Jones presented his group's studies on the impact of baloxavir resistant mutations on influenza A virus fitness. For these studies, they used the active drug baloxavir acid rather than the prodrug baloxavir marboxil. They reconstituted the most common resistant mutation, I38T (conferring 22 to 124-fold loss in potency) and the second and third most common E23 G/K (conferring 2 to 9-fold potency loss), into H1N1pdm09 (A/California/04/2009) and H3N2 (A/Texas/71/2017) seasonal influenza backgrounds. Resistance was synergistic in cultured cells, but the double mutants were also replication impaired. The single mutants had some replication defects, but the effects were not as significant as the double mutants and were most obvious in the H1N1 background. They proceeded to evaluate airborne transmission studies in ferrets. All mutant viruses productively replicated in the inoculated

animals, and the single mutants transmitted well to direct and aerosol contacts. The double mutants were slightly impaired for aerosol transmission, resulting in later infections in not all the exposed animals. Modeling the resistance suggests that the effects of the mutations are indirect, through destabilization of the critical residue Y24. One anchor interaction of Y24 is lost while the second one is intact in mutant E23G, but both are lost with mutant E23K. Destabilization of Y24 is modeled to also result in losing some RNA binding affinity. During the question-and-answer period, Jeremy stated that they are performing a competitive transmission study *in vitro* first and plan to do them *in vivo* later. Regarding to the rationale of including E23K, this mutation is not as frequently identified as I38T, but it has been reported in clinical trials and in children. In brief, PA E23 G/K viruses providing baloxavir reduced susceptibility are capable of infecting and transmitting among ferrets by direct and airborne routes, but other aspects of their fitness are somewhat impaired.

5. Session 2: retroviruses and other viruses

5.1. Cell-to-cell transmission by emerging viruses: mechanisms of action and evasion of Host Immunity. Shan-Lu Liu, M.D., Ph.D., Ohio State University, Columbus, Ohio, USA

Emerging and re-emerging viruses spread globally without borders. While much has been studied on cell-free infection and entry mechanisms of viruses, less is known about the impact of cell-to-cell transmission on the spread, immune responses, and viral pathogenesis. In this talk, **Dr. Shan-Lu** focused on his recent work on SARS-CoV-2 spike-mediated cell-to-cell transmission in the context of other highly pathogenic SARS-CoV, Ebolavirus (EBOV) and HIV-1, as well as in comparison with that of cell-free infection. He provided evidence that the SARS-CoV-2 spike protein is more efficient in facilitating cell-to-cell transmission as compared to the SARS-CoV spike, which reflects, in part, their differential cell-cell fusion activity. Interestingly, treatment of co-cultured cells with endosomal entry inhibitors, including those of cathepsin L essential for cleavage of the SARS-CoV-2 spike and membrane fusion, impairs the efficiency of cell-to-cell transmission, implicating endosomal membrane entry as an important underlying mechanism. Notably, this pattern is like that of EBOV, where cathepsin B/L inhibitors strongly decrease not only cell-free infection but also cell-to-cell transmission. However, different from GP-mediated cell-to-cell transmission of EBOV, which is sensitive to inhibition by a potent human KZ52 antibody, the cell-to-cell transmission of SARS-CoV-2 is refractory to inhibition by neutralizing antibodies induced by mRNA vaccines or convalescent sera from COVID-19 patients. What is the role of viral receptors in cell-to-cell transmission? In the case of HIV-1 and EBOV, they discovered that viral receptors CD4 and NPC1 are important for both cell-to-cell transmission and cell-free infection: CD4 is involved in formation of the virological synapse of HIV-1 Env-mediated cell-to-cell transmission, whereas NPC1 is an endosomal protein required for EBOV GP binding and entry into target cells. Interestingly, although the SARS-CoV-2 receptor ACE2 enhances viral entry and cell-free infection, it is not absolutely required for cell-to-cell transmission of SARS-CoV-2. Notably, despite differences in cell-free infectivity, we find that SARS-CoV-2 variants of concern (VOC) B.1.1.7 (alpha) and B.1.351 (beta) have similar cell-to-cell transmission capability. However, the Omicron variant (B.1.1.529) exhibits significantly increased capability of cell-to-cell transmission in lower airway human airway epithelial cells, which is likely associated with the reduced pathogenesis of Omicron compared to other VOCs, such as Alpha and Delta. Overall, the studies on cell-to-cell transmission of emerging and re-emerging viruses have revealed distinct, sometimes virus-specific and cell type-dependent, effects of viral receptors and cofactors in this important process. A better understanding of the virus cell-to-cell transmission aids in the development of new and better antiviral therapeutics against human and animal diseases.

*5.2. Lenacapavir: the first clinically active long-acting inhibitor of HIV capsid, Christian Callebaut, Ph.D., Gilead Sciences, Inc., Foster City, California, USA**

A program building on prior extensive structural and functional characterization of HIV capsid and spanning a decade of drug discovery work yielded Lenacapavir (LEN, GS-6207), a small molecule inhibitor targeting several critical functions of HIV capsid. LEN binds at a conserved interface between capsid monomers and interferes with protein interactions essential for multiple phases of HIV replication cycle including both assembly and disassembly of capsid core, as well as capsid nuclear trafficking. LEN exhibits antiviral activity at picomolar concentrations against all subtypes of HIV, including strains resistant to other antiretroviral classes, and is amenable to both oral and long-acting subcutaneous administration. In a Phase 1 study in treatment-naïve people with HIV, LEN (50 mg–750 mg) showed a rapid and dose-dependent antiviral effect, with up to 2.3 mean log10 decrease in HIV-1 RNA at day 10. In people with multi-drug-resistant HIV, subcutaneous LEN administered every 6 months in combination with an optimized background regimen led to high rates of virologic suppression and was well tolerated. Finally, emerging data from non-human primates support further investigation of LEN as a long-acting agent for pre-exposure prophylaxis.

5.3. Antiviral therapy for Lassa fever, Lisa Oestereich, Ph.D., Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Lassa fever (LF) is a zoonotic viral infection caused by Lassa virus (LASV), an Old World arenavirus. It is assumed to be responsible for up to three hundred thousand cases and more than five thousand deaths annually, with fatality rates ranging from 20% to 70% in hospitalized patients. LF is endemic in many West-African countries, and its epidemic potential and the lack of therapeutics or vaccines lead to its classification as a priority pathogen by the WHO. Severe LF may be associated with haemorrhage, oedema of the face and neck, liver damage, kidney dysfunction, and central nervous system manifestations such as coma and seizures. But despite the high morbidity and mortality caused by LASV infections, very little is known about the pathophysiology of the disease. Analysis of plasma samples from human LF patients showed that the dysregulation of homeostasis, strong inflammation and vascular dysfunction are hallmarks of LF. High levels of IL-6, IL-8, and IP-10, as well as PAI-1 and thrombomodulin, were notable in all LF patients, with higher levels correlating with fatal outcomes. These findings confirm that pathogenic host immune responses during LF are hyper-inflammatory. Analysis of PBMC revealed that LASV specific and bystander CD8 T cells are activated, and that both activation and exhaustion markers on these cells are upregulated.

To better understand the role of T cells in the pathophysiology of LF and the underlying causes of the in humans often observed multi-organ damage, **Prof. Lisa Oestereich** and her coworkers developed a susceptible mouse model with intact adaptive, hematopoietic-driven immune response. Using transplantation of wild-type bone marrow cells into IFNAR−/− mice, they generated chimeric mice that reproduced key features of severe LF in humans, including high lethality, liver damage and vascular leakage. They used this mouse model to describe the role of T cells during acute infection and observed that T cell-mediated immunopathology plays an essential role during LF. Like the results in humans, they could show that exhaustion markers are upregulated despite a high level of activation of these cells.

Based on these results, they tested different disease-modifying drugs for experimental LF treatments in mice. FX-06, a fibrin-derived peptide, which has been developed to reduce stress-fiber mediated vascular leak, has already been successfully used to treat dengue shock syndrome, Ebola virus disease and is currently under clinical investigation for the treatment of acute respiratory distress syndrome during Covid-19 pneumonia. The treatment of LASV-infected mice with FX-06 alone

abolished the vascular leakage but could not rescue the mice. However, in combination with a subtherapeutic dose of the directly acting antiviral drug Favipiravir, mice could be rescued, even if the treatment start was delayed until the onset of disease symptoms. They also screened a wide range of commercially available immune-modulatory drugs. They could identify two candidates that were able to reduce the disease symptoms to a remarkable degree and treated animals that survived the infection. Sirolimus and Cyclophosphamide reduced the symptoms without affecting virus load in blood or organs. The surviving mice had a significantly diminished T cell response, indicated by a low number of LASV-specific T cells found in these animals. The treatment success with these disease-modifying drugs represents a novel approach to treat LF, targeting the underlying pathomechanisms rather than virus replication directly and might be a good approach for combination therapies with directly acting antivirals.

5.4. Prophylactic treatment with a defective interfering particle-based therapeutic protects hamsters from lethal Nipah virus disease primarily by direct inhibition mechanisms. Stephen Welch, Ph.D., Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Dr. Stephen Welch described the development of a novel treatment platform targeting Nipah virus (NiV), based on defective interfering particles (DIs). DIs are a natural by-product of viral infection, known to alter viral propagation kinetics and exert potent inhibitory properties in cell culture. He described how they characterized naturally occurring NiV strain Malaysia DIs, developed a production system to generate them in large quantities, and then investigated their effectiveness as a treatment in the Syrian hamster model of clinical disease. When DIs were administered prophylactically, they conferred up to 90% survival against homologous virus challenge, and significantly reduced the appearance and length of clinical signs. However, post exposure DI treatment did not exhibit any protective effect against challenge. He explained that the relative contribution of direct (competition between DI and full-length virus for resources) and indirect (non-specific immune responses) inhibition elicited by DIs *in vivo* remains poorly defined. He presented data on the effectiveness of *in vivo* DI treatment against a different strain of NiV (strain Bangladesh) and the closely related Hendra virus, demonstrating that increasing heterogeneity of the challenge virus was associated with decreasing effectiveness of treatment. This observation, coupled with evidence they presented revealing only a mild and transient innate immune response to DI treatment, supported direct interference as the primary mechanism of action for these DIs. He concluded that these data support potential therapeutic applications for DI particles and encourage further development of DI therapeutics for NiV and for other high-consequence pathogens.

5.5. Small molecule antivirals inhibit HuNoV GII.4 in human intestinal enteroids. Nanci Santos-Ferreira, M.S., KU Leuven, Rega Institute, Laboratory of virology & Chemotherapy, Leuven, Belgium

Human Norovirus (HuNoV) is the main cause of acute gastroenteritis resulting in high morbidity and mortality, especially in children under the age of five. On top of that, chronic norovirus gastroenteritis is a common complication in immunocompromised patients. Unavailability of antiviral drugs or vaccines is mostly due to inability to culture HuNoV. Human intestinal enteroids (HIEs) have emerged as a unique opportunity to culture HuNoV *in vitro,* as this virus cannot grow in other immortalized cell lines or primary cultures (Microorganisms. 2021 Jul 27; 9(8):1599). To setup an antiviral assay using HIEs, HuNoV (derived from stool samples of infected patients) replication was evaluated in HIEs derived from two different regions of small intestine: adult jejunum (J2) and fetal ileum (FI124) tissue. HuNoV infection was performed in a) 2D differentiated enteroid monolayers in collagen-coated plates; and b) 3D differentiated enteroids in matrigel. Viral loads were quantified by RT-qPCR at day 2 or 3 post-infection (pi) and compared to cultures harvested 1 or 2 h pi. Replication of HuNoV GII.4 strains reached higher yields in the FI124 line and when infection was performed in 3D, attaining an increase over input of 2.5 $\log_{10}$ in HuNoV viral RNA copies. HuNoV infection of HIEs was confirmed by immunofluorescence using antibodies that target viral capsid (VP1) and dsRNA, showing that we have active replication in the enteroids. To validate the infection model for antiviral drug discovery, infected enteroids were treated with known antivirals active against noroviruses and/or other RNA viruses. The cytotoxicity of compounds was also assessed using the CellTiter-Glo 3D cell viability assay. Treatment of HuNoV GII.4 3D infected FI124 enteroids with the polymerase inhibitor 2′-*C*-Methylcytidine (2CMC) showed the expected dose-depend antiviral activity with a reduction of 1.9 $\log_{10}$ in HuNoV viral RNA copies when treated with 200 μM after infection. Other antivirals that showed activity in this model include favipiravir, molnupiravir, rupintrivir and nitazoxanide. The active metabolite of nitazoxanide, tizoxanide, was also tested, resulting in the same levels of inhibition and confirming the proper metabolization of the compound. Overall, 3D infection of FI124 HIEs was shown to be a robust model to evaluate antivirals that will now be used to assess the antiviral activity of novel compounds against HuNoV GII and move forward the antiviral research on HuNoV to better manage norovirus disease.

*5.6. Monoclonal antibodies against both flu and RSV. George F. Gao, Ph. D., Institute of Microbiology, Chinese Academy of Sciences, Beijing, China**

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

6. Session 3: broad spectrum antiviral drugs and pandemic preparedness

6.1. Out of the box: 20 Years of targeting virus infections unconventionally. Marco Vignuzzi, Ph.D., Institut Pasteur, Paris, France

Prof. Marco Vignuzzi began by announcing the move of his lab to the new ID Labs within the Agency for Science, Technology and Research in Singapore. He continued by covering a history of creative and self-described “impractical ways” to target viruses, beginning in the late nineties as a PhD student working on RNA vaccines, a vaccine

approach that was largely dismissed at the time. Afterwards, he developed a novel approach for mucosal delivery of RNA using bacteria (salmonella with gene knockouts). In the years that followed, the work in Marco's lab capitalized on antiviral research to drive RNA mutagen resistance studies to find variants, and in turn address basic research questions on the role of mutation rate and virus fidelity in virus adaptation. This work included identification of strains that can be used for vaccines, i.e., strains less likely to revert, and the study of how other molecules can affect replication fidelity (e.g., Amiloride). They looked at both high and low fidelity variants and found that, in general, viruses do not like to move away from their inherent level of fidelity. Work on virus fidelity continued with use of biological and biochemical data; they found that polymerase speed and fidelity are correlated, and fidelity and recombination are correlated.

Marco's lab applied antiviral research creatively; panels of virus variants were constructed based on exposure to compounds, and sequencing approaches were applied to follow evolution and gain critical information from low level changes, essentially monitoring for the emergence of variants before they happen. He touched on a new generation of live virus vaccines based on their work developing viruses that looks like wildtype but are prone to mutations, specifically the introduction of stop mutations so that infection results in short-lived replication in the host due to the introduction of stop codons; and work showing that like DNA viruses, polyamines are required for RNA viruses.

Marco finished his talk by presenting their most recent work looking at defective viral genomes (DVGs) as part of the DARPA INTERCEPT program. They have developed evolutionary and computational approaches to generate DVG candidates by serial passaging and used fitness measures to shortlist the best candidates and identify deletion hot spots. Interestingly, high fitness DVGs were found to outperform classic shorter DVGs. In mice, DVGs could reduce virus titers, and were detected in tissues both proximal and distal to the footpad delivery site, including brain and ovaries several days later. DVGs were then evaluated in mosquitoes and was found to both inhibit virus by RNAi-dependent mechanism and block transmission of virus. Finally, using CHIKV as an example, DVGs were found to be broadly acting within a virus family.

*6.2. The SARS-CoV-2 main protease: new inhibitors, crystal structures, and mutations. Rolf Hilgenfeld, Ph.D., University of Luebeck, Institute of Molecular Medicine, Luebeck, Germany**

In February 2020, we determined the crystal structure of the SARS-CoV-2 M^{pro} and presented a powerful alpha-ketoamide inhibitor, compound 13b [Zhang et al. Science, 2020]. Using a structure-based approach, we have since optimized this compound further and now have inhibitors with IC50 down to 13 nM in the biochemical assay and EC50 $<$ 400 nM in virus-infected cell culture. ADME and pharmacokinetic data will be discussed for the frontrunner compounds. In preparing for future drug resistance mutations, which will likely emerge when the presently available SARS-CoV-2 M^{pro} inhibitor nirmatrelvir and potential future candidate compounds such as 13b-K will be used in the clinic, we analyzed the natural evolution of the M^{pro} since the beginning of the pandemic. When cumulated, the most common mutations are L89F and K90R, and we determined crystal structures for both. However, the frequency of L89F is declining, and others are coming up; and we will also present structural studies on the most interesting ones. Most mutations are far from the substrate-binding site and the dimerization interface of the enzyme, and the inhibitory potency of compound 13b-K is not affected. The M^{pro} mutation P132H is characteristic for the Omicron variant of concern of SARS-CoV-2, and we will present data on this protein as well. This work was supported, in part, by the BMBF (directly and through DZIF) and by the European Commission.

6.3. Host targeting broad-spectrum antivirals for pandemic preparedness. Jeffrey Glenn, M.D., Ph.D. Stanford University School of Medicine, Stanford, California, USA

Dr. Jeffrey Glenn began by detailing the benefits of host-targeting antivirals for pandemics, including high barriers to resistance, broad-spectrum potential, and additional functionality and relevance in non-viral diseases. His first example was targeting host farnesyl-transferase to combat hepatitis D virus (HDV), using lonafarnib (LNF) which inhibits attachment of the prenyl lipid farnesyl to the large delta antigen of HDV. He presented data showing that LNF serum concentration correlates with viral load decline; and through a series of phase 2 studies, they found an optimal combination of LNF with ritonavir (RTN) with or without IFN-alpha to treat HDV. Importantly, he also revealed that these combinations showed no evidence of the development of resistance. These data led to the first-ever phase 3 registration study in HDV called Delta-Liver Improvement and Virological Response in HDV (D-LIVR), with first results expected by the end of 2022.

Jeffrey then moved on to discussing the dependance of hepatitis C virus (HCV) replication on a host intracellular lipid, derived from phosphatidylinositol (PI) by the actions of PI 4-kinases (PI4K). This interaction was localized to a basic amino acid PIP2 pincer (BAAPP) domain, with similar domains found in a variety of other pathogens including enteroviruses, rhinoviruses, ebola virus, and SARS-CoV-1 and SARS-CoV-2. He presented data with the PI4K-inhibitor STF-1019, showing dose-dependent protection from death in a lethal mouse model of enterovirus EV71. In a continuation of this work, the optimized lead molecules now exhibit picomolar EC_{50} values and are active against multiple viruses including EV68, HRV, Ebola, SARS-CoV-2. They have also shown potential for non-viral indications, with *in vivo* activity against lung cancer, and triple negative breast cancer.

Jeffrey next presented data on the broad-spectrum antiviral activity of pegylated interferon lambda treatments, discussing the potential for combination therapy with other molecules. Building on data presented earlier, he introduced the lambda interferon combination therapy (LIFT) study, which evaluated the effect of replacing interferon-alpha with interferon-lambda in a phase 2 study treating HDV with LNF + RTN + IFN. As predicted, it was better tolerated than IFN-alpha therapy with comparable efficacy, with 77% achieving the primary endpoint of a greater than 2 log decline in HDV RNA at study end, and 23% demonstrating durable virological response at 24 weeks post-treatment. He concluded with data showing interferon-lambda is broadly active against numerous viruses *in vitro* and in animal models (e.g. SARS-CoV-2, influenza, etc.), and has a well-established tolerability profile in over 3000 patients in the context of over 20 clinical trials. Studies have indicated that interferon lambda therapy is highly effective against SARS-CoV-2, which has led to phase 2 and 3 studies, with a large phase 3 study showing unparalleled efficacy following a single subcutaneous dose.

6.4. Efficient incorporation and template-dependent polymerase inhibition are major determinants for the broad-spectrum antiviral activity of remdesivir. Calvin Gordon, Ph.D. Candidate, University of Alberta, Edmonton, Canada

Calvin Gordon presented data from biochemical studies on the mechanism of action of remdesivir (RDV), showing it acts at the molecular level against the polymerases (RdRps) of different RNA viruses. He began with data showing that SARS-CoV-2 RdRp incorporates the triphosphate form of RDV (RDV-TP) more efficiently than the natural nucleotide (ATP). He then showed that, with SARS-CoV-2 polymerase, RDV-TP incorporation during 1st strand synthesis at position *i* results in delayed chain termination at position *i*+3. A strong correlation is observed between antiviral effects and efficient incorporation of RDV-TP. In comparing effectiveness across virus families, Calvin showed that, compared to positive sense viruses, RDV was still effective against

non-segmented negative sense ssRNA viruses (EBOV, RSV, NiV), but less effective against segmented negative sense ssRNA viruses (LASV, CCHFV, influenza). He further presented data showing that delayed chain termination during polymerase primer extension (1st stand synthesis) is often not very effective and heterogenous amongst the different polymerases. In contrast, template-dependent inhibition (2nd strand synthesis) was uniform across multiple polymerases evaluated (SARS-CoV-2, HCV, and CCHFV), with molecular modeling suggesting a steric conflict between the 1′-cyano group of RDV-MP and conserved residues of RdRp motif F. Finally, he showed that for SARS-CoV-2, multiple embedded RDV-MP residues potentiate template-dependent inhibition. Calvin concluded that future drug development efforts that aim at a broader spectrum of antiviral activities could focus on improving rates of RDV incorporation while still capitalizing on the inhibitory effects of a bulky 1'-modification.

6.5. Countering pathogenic new world mammarenavirus infections through receptor-targeted disruption of virus entry. Brian Gowen, Ph.D., Utah State University, Utah, USA

Prof. Brian Gowen summarized the disease burden and geographic range of the pathogenic arenaviruses, including LASV, and Lujo viruses (both old world mammarenaviruses), and Machupo (MACV), and Junin (JUNV) viruses (both new world mamarenaviruses). He referred to previous studies that showed that transferrin receptor 1 is a major cellular receptor of new world mammarenaviruses (NWM), and how an antibody recognizing this receptor, ch128.1, was able to efficiently inhibit the entry of pathogenic NWMs. However, due to the inherent costs involved with NHP studies to investigate *in vivo* efficacy of this antibody (and others like it), research on these antiviral options faltered. To overcome this, he described how his lab developed a small rodent model of arenavirus infection, based on challenge of mice expressing human transferrin receptor 1 (hTfR1). JUNV infection in these mice resulted in severe and lethal disease. Brian then presented data showing that treatment with the ch128.1/IgG1antibody in this model protected the mice against lethal JUNV strain Romero challenge, with an Fc-silent mutant version of ch128.1 offering significantly more protection than the standard version of the antibody. He presented computational docking data of the antibody Fab crystal structure onto the known structure of hTfR1, showing an overlapping receptor-binding region shared by the Fab and the viral envelope glycoprotein GP1 subunit of MACV that binds hTfR1. He concluded his presentation with data proving that the Fab competes with immobilized MACV GP1 binding to soluble TfR1, demonstrating competitive inhibition of NWM GP1 binding by ch128.1/IgG1 as the principal mechanism of action. He concluded that these promising preclinical data support continued development of novel, host receptor-targeted antibody therapeutics broadly applicable to the treatment of hemorrhagic fevers of NWM etiology.

6.6. Development of small molecule entry inhibitors as potential filoviral therapeutics. Laura Cooper, Ph.D., University of Illinois at Chicago, Chicago, Illinois, USA

Prof. Laura Cooper started by describing filovirus entry mechanisms, focusing on a class of inhibitors believed to be fusion inhibitors, and then the pseudovirus system that they used to study viral entry of this BSL-4 pathogen under the lower BSL-2 containment. She explained the screen of an FDA-approved library carried out during the 2013–2106 West African EBOV outbreak that identified toremifene as an inhibitor of both EBOV and MARV entry, describing a binding pocket at the GP1/GP2 interface which destabilizes the EBOV protein which is the potential mechanism of inhibition. To assess whether this binding was functional, Laura's group performed mutational analysis of residues in this pocket and discovered that one mutation (Y517S) generated a MARV-like inhibition curve rather than completely ablate the activity. This result led them to consider that there may be a secondary mechanism leading to the pan-filovirus activity demonstrated by toremifene. An overlay of the EBOV and MARV glycoproteins showed that in the GP1/GP2 interface for MARV there was an α-helix (α2), making it unlikely that small molecules could access that binding pocket. To confirm this hypothesis, they mutated further residues in the active site of the EBOV-GP, and they saw the same MARV-like phenotype for more than just the Y517 residue, providing further evidence of a separate mechanism of action for EBOV and MARV inhibition. She then presented data with the toremifene analog ospemifene, which suggested that the terminal amine of toremifene is relevant to the secondary mechanism of inhibition. Direct binding studies confirmed that toremifene binds to the MARV-GP, which lead them to believe there was a binding pocket on this protein that was distinct from the analogous GP1/GP2 binding pocket previously described for EBOV. Laura went on to describe studies that identified the HR2 region of the GP as a possible conserved secondary pocket. She concluded her presentation by describing synergy studies using toremifene and another small molecule that only binds to the HR2 region, fluoxetine, suggesting that synergism can be achieved by targeting each GP pocket on the EBOV-GP. This synergy was not apparent for MARV as it is believed to only have the HR2 pocket. This work lays the foundation to guide the design of novel pan-filovirus entry inhibitors.

6.7. A photoactivable chlorophyll-derived product with broad antiviral activity against enveloped viruses including highly pathogenic coronaviruses. Karin Séron, Ph.D., Center for Infection and Immunity of Lille, Lille, France

Dr. Karin Séron began her presentation with a question: is it possible to identify anti-coronavirus agents in medicinal plants from Côte d'Ivoire? She described *in vitro* antiviral activity analysis of 15 crude Ivorian plant extracts, from which they identified *Mallotus oppositifolius* as the most active compound against HCoV-229E. Using bio-guided fractionation of this extract, they further identified pheophorbide a (Pba) as a potent antiviral molecule and demonstrated this compound's antiviral activity against the highly pathogenic coronaviruses SARS-CoV-2 and MERS-CoV in cell culture. Given that Pba is a light-activated molecule, they next investigated whether the mechanism of action was light activated. Karin presented data showing that the antiviral activity of Pba depended on light exposure, and on the generation of singlet oxygen, revealing a photodynamic inactivation mechanism. She next discussed data revealing the mechanism of action as a coronavirus entry inhibitor was by directly targeting the viral particle, using cryo-electron microscopy to demonstrate Pba-mediated stiffening the viral membrane which prevents the deformation needed to undergo virus-cell fusion. She concluded her presentation describing how Pba was broadly active against several other enveloped viruses (HCV, SINV, YFV), but not against the non-enveloped CVB4.

6.8. A broad-spectrum ribonucleoside analog, EIDD-2749, provides protection against enterovirus D68 and 71 infections in mouse models. E. Bart Tarbet, Ph.D., Utah State University, Utah, USA

Prof. Bart Tarbet began his presentation by describing the most common symptoms due to enterovirus A71 (EV-71), the causative agent for hand, foot, and mouth disease in humans which also has a poorly characterized neurological involvement in 5–30% of cases. To better characterize this neuropathogenesis, he described the development of a mouse model for an EV-71 strain originally passaged in mice 4 times, but then adapted to AG129 mice by a further 6 sequential passages to obtain consistent lethality. Infected mice developed viremia with rapid tissue distribution, as well as paralysis and weight loss. He also described the neurological scoring system used to characterize the clinical effect of infection. He then described a study where they assessed the efficacy of EIDD-2749, a ribonucleoside analog, in this newly developed mouse model. Using a delayed treatment study where mice were treated daily

either 3-, 5-, or 7-days post challenge with 10 mg/kg of EIDD-2749, all treated groups scored significant less using the neurological scoring system compared to the placebo control, with 100% survival at all time points. Bart went on to describe the development of a respiratory model for EV-D68 in AG129 mice (adapted by 30 sequential passages). He presented data on a prophylactic study in this second model, with EIDD-2749 treatment starting 2 h prior to challenge and continuing twice daily for 5 days. On this study, he showed that mice had significantly reduced virus titers in their lungs at 1-, 3-, and 5-days post challenge, with a reduction in pro-inflammatory cytokines also noted. With a similar treatment regimen, mice treated with the highest dose of EIDD-2749 (60 mg/kg) also presented with reduced neurological scores with 100% survival.

6.9. Antiviral activity of geneticin against SARS-CoV-2. Gregory Mathez, M.S., University Hospital of Lausanne and University of Lausanne, Lausanne, Switzerland

Gregory Mathez began his presentation introducing geneticin, an aminoglycoside antibiotic known to interact with ribosomal RNA. Geneticin has shown broad-spectrum activity against RNA viruses, in particular hepatitis C virus, through interaction with double-stranded tertiary structures in the viral RNA. He presented data showing that geneticin inhibited all tested variants of SARS-CoV-2 at non-toxic concentrations *in vitro* in Vero cells. To understand antiviral efficacy in a more representative model, he used Calu-3 cells (human epithelial lung cells) and showed a dose response with geneticin against the B.1.1.7 Alpha variant. Gregory then presented data using the MucilAir™ ex vivo cell model of the human airway epithelium, showing that treatment with geneticin 24 h post infection with either the ancestral SARS-CoV-2 or Omicron strain resulted in a reduction in virus levels. He then showed data investigating the mechanism of action, showing that geneticin binds a tertiary pseudoknot structure of viral RNA responsible for the −1 programmed frameshift of SARS-CoV-2, and acts rapidly to decreases the levels of both viral protein and viral RNA. Gregory concluded his presentation by describing a virtual screening study he performed to identify analogs of geneticin with increased potency and conserved activity, but with potential less side effects.

6.10. Automated brightfield microscopy and AI to develop rapid high throughput infectivity assays for screening antiviral drugs and monitoring vaccine effectiveness against emerging variants. Ilya Goldberg, Ph.D. ViQi Inc, Santa Barbara, California, USA

Dr. Ilya Goldberg began his presentation by describing the 2020 NSF call for proposals to develop innovative technologies to address the COVID-19 epidemic, asking the question "can we detect virus infection on brightfield images?" He showed that many viruses produce membraned structures, including double-membraned vesicles (DMV) and replication sites, of around 400 nm in size, and argued that these membrane alterations should have a readout using phase contrast or brightfield microscopy techniques. To detect these alterations, Ilya's company, ViQi, Inc., has developed a quantitative viral infectivity assay using machine learning and brightfield microscopy to identify these phenotypic changes within cells. He presented data showing that these techniques were able to detect alterations associated with viral production before they were humanly visible, dramatically reducing the incubation times associated with standard viral infectivity assays such as plaque-, TCID50-, and fluorescent labelling-assays. He described the fully automated AI-training of an automated viral infectivity assay (AVIA) which can detect infection in less than 24 h, presenting data showing that AVIA works with serval virus species regardless of their infectivity pathways, including HIV, influenza, vaccinia, polio, and adenoviruses. Ilya concluded his presentation discussing how, since this technology relies on AIs sensitized to potentially specific phenotypes of viral production, it may have potential as a rapid, broad-based identification and diagnostic tool.

7. Session 4: arboviruses

7.1. Antiviral strategies for arboviruses: a 'buzzing' role for the mosquito vector? Leen Delang, Ph.D., University of Leuven, Leuven, Belgium

The focus of **Prof. Leen Delang**'s talk was on the opportunities to control arboviruses through the effects of antivirals on mosquitoes. Leen highlighted that mosquitoes are the deadliest animals in the world, through the diseases they transmit (parasites and viruses). Three arboviruses, chikungunya, dengue, and Zika viruses are endemic in most tropical countries. Often two or three of them overlap in the same geographical area, and six billion people are at risk of being infected at least once a year with one of them. Mosquito borne viruses produce very high morbidity; for example, about 50% of the people infected with chikungunya virus develop chronic disease. Mosquito borne viral infections are not only of concern in equatorial or warm regions of the world. For example, Culex mosquitoes, which are important viral vectors transmitting for example WNV, are distributed through temperate countries.

Leen used a blank slide to illustrate "all" the FDA approved drugs against arboviruses, but she also highlighted that NS4B dengue inhibitors are advancing in the pipeline. She discussed JNJ-A07, an NS4B pan serotype dengue inhibitor discovered at the Rega Institute by Johan Neyt's group, which is active *in vivo* and was in phase II clinical trials. There are nonetheless intrinsic challenges for antivirals against these viruses. For example, the ideal treatment window for a dengue inhibitor should include the time when it can lower viremia, to minimize further infection of mosquitoes and thus control dissemination. However, the level of viremia to infect a mosquito depends on the virus and mosquito, and viremia lasts for just a week at most. Early diagnostics are required to allow for this use of antivirals to lower viremia, but diagnostics are challenging because of the overlap of clinical symptoms among different viruses, and even with malaria. An alternative would be to use the inhibitors as pre-exposure prophylaxis for travelers or household contacts. In this use, an antiviral drug will be ingested by a mosquito biting the treated and yet uninfected person. As ivermectin had been proven to have mosquiticidal activity when the mosquitoes bite a person under treatment, Leen's group is focused on evaluating whether antiviral drugs could have similar mosquiticidal activity or have antiviral activity in mosquitoes. To this end, they use mosquito cells and adults and have furthermore developed an in between model, an *Aedes aegypti* mosquito midgut model. Dissected midguts are explanted and remain viable for about 6–10 days, even maintaining peristalsis. These explants can be infected with alphaviruses and flaviviruses, which are transmitted by Ae aegypti, but not with Usutu virus, which is transmitted by Culex. The model simplifies biohazard management as no live mosquitoes are infected.

Using these models, they have tested several antivirals. Favipiravir had no activity in mosquito cells in concentrations up to 200 μM, most likely because it needs to be metabolized by HGPRT, which is not expressed in insects. Indeed, favipiravir was metabolized in Vero cells to mono, di, and tri-phosphate forms, but not in mosquito cells. Favipiravir had also no antiviral effect in adult mosquitoes. They also tested JNJ-A07, which does inhibit replication in the mosquito adult (Ae aegypti). High doses of 100 μM prevented dissemination of the virus to the head. This is a new area of research, and consequently, there is still only limited information on these models. For example, PK in mosquitoes is unknown, as is whether the antiviral drug may have any effect on already infected mosquitoes. It is also unknown whether resistance may be selected for in the mosquitoes. Conversely, they have tested chikungunya viruses resistant to two different drugs, favipiravir and MADTP, a capping inhibitor. MADTP-resistant strains were efficiently transmitted by mosquitoes whereas favipiravir resistant ones were not. It was unknown if any antivirals are absorbed through mosquito leg

cuticle (tarsal exposure), as was previously shown for atovaquone. Leen's group found that tarsal exposure to atovaquone resulted in very small effect on CHIKV titers in the heads. Furthermore, no infectious virus was detected in the saliva, in contrast to detection in the saliva in 15% of the untreated control mosquitoes, suggesting a modest antiviral effect of atovaquone via tarsal exposure. In summary, Leen's talk discussed the analyses of the activities of antivirals in mosquito vectors.

*7.2. New insights into cellular infection by the mosquito-transmitted Rift Valley Fever Virus. Amy Hartman, Ph.D., University of Pittsburgh, Pittsburgh, Pennsylvania, USA**

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

*7.3. NITD-688, a pan-serotype inhibitor of the dengue virus NS4B protein, shows favorable pharmacokinetics and efficacy in preclinical animal models. Feng Gu, Ph.D., Novartis Institute for Tropical Diseases, Emeryville, California, USA**

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

7.4. AI-derived antibody discovery – humanoids for global good. Randal Ketchem, Ph.D., Just – Evotec Biologics, Seattle, Washington, USA

Dr. Randal Ketchem spoke on AI-derived antibody discovery. He started the presentation describing the mission of Just, which is to design and apply innovative technologies to dramatically increase global access to biotherapeutics. Just is based in Washington and is active in the antiviral development process from discovery to manufacturing. The talk focused on the use of artificial intelligence to discover antibody-based therapeutics that can quickly be fed into a development platform. The AI approach used is based on generative adversarial networks (GAN), which results in a sequence generator able to produce developable human antibody sequences. The system does not know the first principal attributes of human antibody sequences or developability, but is able to train and transfer learn these properties. To generate therapeutic biologicals, the approach used a very large number of sequences of human B-cell antibody repertoire from the OPIG OAS set, removing recently immunized or convalescing patients. The system generates a variety of novel human antibodies that are synthesized as Fab fragments presented as phage display libraries. This approach was selected as it results in similar sensitivities of the expressed proteins to that of natural antibodies, making the platform suitable for discovery and ultra-high throughput developability characterization. Examples of transfer learned properties under development are in efficacy diversity such as broad variation in HC-CDR3 and surface properties, and developability properties such as temperature, pH, and chemical denaturant stability, titer, and self-interaction. He then introduced an example of the application of the technology to SARS-CoV-2 neutralizing antibodies, keeping the focus on creating antibodies optimized for developability. During the question-and-answer period, the challenges for AI with the large data and noise in the antibodies sequences was discussed. However, the Ig fold is fairly stable in the core fold, and the training includes millions to billions of data points, maximizing the robust nature of the process. This talk highlighted an innovative approach toward antiviral therapeutic development.

7.5. Dengue virus infection and dissemination in aedes mosquitoes is significantly reduced upon exposure to JNJ-A07, a potent DENV inhibitor, in the blood meal. Ana Rosales Rosas, M.S., Rega Institute for Medical Research, KU Leuven, Leuven, Belgium

Ana Lucia Rosales expanded on the earlier presentation by Leen Delang on JNJ-A07. In her talk, Ana described the effects of JNJ-A07 on mosquitoes and DENV infection in detail. JNJ-A07 had no effect on mosquito lifespan but egg development was slightly reduced by 1.2-fold. Mosquitoes were next infected with a bloodmeal containing dengue virus and spiked with JNJ-A07. Doses of 2 and 25 μM, selected from the PK in mice, fully inhibited mosquito infection or dissemination. There was still significant inhibition at a 10-lower dose of 0.2 μM, but the antiviral activity started to vanish at 0.02 μM. Although they had yet to explore pre- and post-infection treatments, these results indicate that mosquito exposure to JNJ-A07 in the blood ingested from a treated person may contribute to the management of a dengue outbreak.

*7.6. AT-752, a double prodrug of a guanosine nucleotide analog, is effective against yellow fever virus in a hamster model. Abbie Weight, B.S., Institute for Antiviral Research, Utah State University, Logan, Utah, USA**

Yellow fever virus (YFV), a re-emerging mosquito-borne flavivirus, causes significant morbidity and mortality in Africa and the Americas. An effective vaccine is available, yet there are no antivirals available to treat the disease. AT-752 is an orally available double prodrug of a guanosine nucleotide analog. Previous research showed that the free base of AT-752 effectively inhibits YFV *in vitro*, with a 90% effective concentration (EC90) of 0.15 μM. We evaluated the *in vivo* efficacy of AT-752 against YFV in a hamster model. Administration of 1000 mg/kg/d AT-752, initiated 4 h pre-infection or 2 days post infection (dpi) and continuing for 7 days, significantly improved survival, viremia, and serum alanine aminotransferase (ALT) levels in YFV-infected hamsters. AT-752 treatment initiated 4 h before viral challenge resulted in significantly reduced viremia and ALT to levels similar to those of sham-infected animals. Treatment initiated 2 dpi resulted in a >2log10-fold decrease in viremia and a 53% decrease in serum ALT ($p < 0.001$ for both) when compared with placebo controls. AT-752 treatment significantly improved survival regardless of dose or time of treatment initiation. Finally, a pharmacokinetic study in hamster tissues found the highest levels of the active metabolite in the kidney and liver, the organs most affected by YFV. These results show that AT-752 has efficacy *in vivo*

against YFV and thus justify further research into the clinical potential of this compound as a treatment for YFV.

7.7. Guanine quadruplexes in the RNA genome of the tick-borne encephalitis virus: a new antiviral target. Ludek Eyer, Ph.D., Veterinary Research Institute, Brno, Czechia

Prof. Ludek Eyer presented guanine quadruplexes in the RNA genome of the tick-borne encephalitis virus as a new antiviral target. Despite the medical importance of tick-borne encephalitis virus (TBEV), with a high number of patients infected with the TBEV suffering of neurological involvement and high mortality rates, there is no antiviral drug against this virus. Fortunately, an effective vaccine has been developed and is recommended in endemic areas.

RNA genomes have complex structure, including the potential to form G-quadruplexes (G4s). There are different types of G4s, parallel, antiparallel, hybrid, intramolecular (unimolecular), or intermolecular. A search for putative G4s in the TBEV genome resulted in the identification of seven potential G4s. All of them are somewhat conserved among flaviviruses, but one showed a high degree of conservation among all TBEV strains. This sequence forms a parallel unimolecular G4 and interacts with G4-binding ligands, as demonstrated by a broad panel of biophysical methods. This G4 in TBEV genome was mutated using a site-directed mutagenesis approach, to stabilize, destabilize, or highly destabilize the G4 formation to evaluate viral RdRp polymerase activity through these sequences and assess viral phenotype. The stabilizing mutation inhibited viral RNA synthesis, and the destabilizing mutations had either no effect or increased RNA replication. Consistent with these biochemical results, the stabilizing mutation decreased fitness of the mutated virus at any multiplicity of infection, whereas the destabilizing mutations showed no effect on viral reproduction capacity. The stabilized mutant was also very sensitive to G4 binding ligands. Interestingly, the highly destabilized G4 mutant promptly reverted to the wild type sequence, whereas the stabilized G4 mutants showed lower reversion rate.

The issue of how to achieve specificity as antiviral with a G4 ligand was discussed in the question-and-answer period, as G4 ligands tend not to have specificity for one particular G4 quartet (they cannot distinguish viral and host G4s). Ludek then highlighted that one G4 binding ligand was in clinical trials against cancer.

*7.8. In Situ click chemistry applied to bunyavirales: from conventional drug design to enzymes assembling their own inhibitors like LEGOs®. Laura Garlatti, M.S., Aix-Marseille Université, Marseille, Bouches-du-Rhône, France**

With a worldwide repartition and limited therapeutic options reported, neglected *Bunyavirales* viruses represent a major public health issue. The replication machinery of these viruses is governed by the intricate L-protein that displays RNA-dependent RNA-polymerase activity (RdRp) and endonuclease (EndoN) activity in its N-terminal end. This key protein, responsible for the cap-snatching mechanism that allows the viral transcription, was identified as a promising target to develop pan-genus antivirals. Its catalytic mechanism of RNA hydrolysis, mediated by $Mg2+$ ions, enables the development of diketo-acids (DKAs) metal-chelating inhibitors. Recently, new rational drug-design concepts have emerged focused on Target-Guided-Synthesis (TGS), a powerful method that directly involves the target that assembles its own inhibitors *in situ* like LEGOs®. Herein, we describe the use of *Bunyavirales* EndoN active sites as reaction vessels for the *In Situ* generation of their own highly specific metal-chelating inhibitors. DKA anchor molecules bearing an azide moiety were synthesized using an optimized pathway and EndoNs affinity towards them was assessed using TSA and MST. The *In Situ* Click Chemistry experiment was designed in 96-well plates using biochemical conditions and various combinations of DKA-azide and alkyne fragments to produce 1,4-triazolyl-diketo-acids (NT-DKAs) in the presence of the enzyme. Hit molecules resulting from this fragment-based screening are identified by HPLC-MS and are synthesized on large scale to enable their full biological characterization.

8. Session 5: coronaviruses

8.1. Pyrimidine inhibitors synergize with nucleoside analogs to block SARS-CoV-2. Sara Cherry, Ph.D., University of Pennsylvania, Philadelphia, Pennsylvania, USA

Prof. Sara Cherry talked about her group's work over the past 2 years since the beginning of the COVID-19 pandemic. As an RNA virus lab, her group readily shifted to SARS-CoV-2 research, using high-throughput screening of compound libraries to identify broad-acting, direct-acting, or host-targeted compounds. Overall, compounds were screened against live virus for potency and efficacy, assessed in primary respiratory cells, investigated to elucidate mechanisms of action, and evaluated in synergy assays. Top candidates were advanced to *in vivo* preclinical testing in collaboration with other labs. In particular, synergy between identified nucleoside biosynthesis inhibitors and molnupiravir or remdesivir were assessed in potency assays, and striking synergy in inhibiting several viral variants was observed. The group also used a qPCR-based assay, air-liquid interface (ALI) cultures, and mice to examine synergy between molnupiravir and dihydroorotate dehydrogenase (DHODH) inhibitors, concluding that pyrimidine biosynthesis inhibitors synergize with nucleoside analogs to block SARS-CoV-2 infection. This work suggests that two activities, reduced replication and inflammation, contribute to the observed efficacy. Sara also presented her group's work capitalizing on the role of microbial ligands/PAMPs to block infection. In particular, STING agonists were found to confer protective efficacy both *in vitro* and in mouse models. Finally, Sara discussed work revisiting the role of entry pathways (cathepsin-mediated and TMPRSS-2 activation-mediated) and described how targeted antivirals may be used to block entry pathways. A VSV system was used to identify additional compounds (e.g., UK-371804, a serine protease inhibitor that also target TMPRSS-2-mediated entry). This work was based largely on the recurring observation that compound efficacy was greatly affected by the cell type used due to differences in pathways utilized by the virus in specific cells, emphasizing the importance of cell selection in early screening efforts.

8.2. Mechanochemistry and drug targeting of the SARS-CoV-2 replication-transcription complex from a single molecule perspective. David Dulin, Ph.D., Vrije Universiteit Amsterdam, Netherlands

Prof. David Dulin discussed work on the SARS-CoV-2 replication-transcription complex (RTC) from a single molecule perspective. It is difficult to investigate RTC function with classic bulk biochemistry because multiple complexes are present in the assay. David presented a high-throughput magnetic "tweezers" apparatus for single molecule biophysics, a technique that can observe each complex to aid in finding drug targets and novel antiviral drugs. The approach allows David's group to monitor elongation dynamics in real time with nearly single base resolution on a ~1 kb long template in the presence of all NTPs.

The assay was applied to reveal the nucleotide addition cycle of SARS-CoV-2 core RTC. The group investigated the biochemical origin of the short- and long-lived pauses that were observed during this cycle. The pauses were not linked to factor exchange, but rather were intrinsic to the RNA polymerase nucleotide addition cycle. David revealed that the short pauses were found to be catalytically competent pauses that are parallel to the nucleotide addition burst pathway, and then went on to describe work characterizing the force dependence of the cycle. Overall, this work identified three catalytic pathways: (1) nucleotide addition burst; (2) slow nucleotide addition pathway; and (3) very slow nucleotide addition pathway. The second part of David's talk focused on investigating the mechanism of action of nucleotide analogs, with a

focus on 3'-dATP and remdesivir-TP. The group found that each of the three catalytic pathways can incorporate specific nucleotide analogs. Remdesivir was found not to act as a terminator; instead, it induces pauses and is incorporated via the very slow nucleotide addition pathway. David concluded the talk by discussing work on viral co-factors, focusing on the role of NSP13-helicase. His group performed the first functional investigation of NSP13-helicase in complex with RTC and found that the NSP13-helicase is key in RTC elongation through secondary structures.

8.3. Profile of PBI-0451 an orally administered 3CL protease inhibitor of SARS-CoV-2 for COVID-19. Uri Lopatin, M.D., Pardes Biosciences, Carlsbad, California, United States

Dr. Uri Lopatin discussed the origin of Pardes Biosciences and work on PBI-0451, a selective, orally bioavailable small molecule. The coronavirus main protease (M^{pro}) was a preferred target for Pardes for several reasons: it is an early viral life cycle target and is conserved across coronaviruses, suggesting relatively limited evolution over time. PBI-0451 was found with the help of virtual screening and computational analyses of the topography of the 3CL M^{pro} active site across multiple pathogenic coronaviruses. Key conserved ligand interactions were identified, and a consensus binding pocket was developed for the design of reversible-covalent inhibitors with pan-coronaviral activity. In biochemical assays PBI-0451 was shown to have broad antiviral activity against a panel of proteases from multiple pathogenic human coronaviruses. Across multiple cell-based assays, antiviral activity in the absence of notable cytotoxicity was seen against SARS-CoV-2, including SARS-CoV-2 variants, with inhibition of replication of all SARS-CoV-2 variants evaluated. Serial passaging in cell culture identified limited resistance. Assessments in the murine model were challenging due to poor pharmacokinetics in mice; however, reduced viral loads and preservation of lung function were noted with both daily and twice-daily dosing. No relevant adverse findings were observed in GLP toxicology studies, and PBI-0451 demonstrated oral bioavailability in all species tested. Uri finished by discussing the first-in-human Phase 1 clinical study in which PBI-0451 has been generally well-tolerated and added that it has demonstrated favorable pharmacokinetics in the phase 1 clinical trial. Phase 2/3 trial is anticipated to begin later this year, informed by dosing regimen information generated in ongoing studies.

8.4. Strategies to interfere with nucleotide excision by the 3'-to-5' exoribonuclease from SARS-CoV-2 Jamie Arnold, Ph.D., University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Dr. Jamie Arnold discussed the role of SARS-CoV-2 ExoN in counteracting antiviral efficacy by excising antiviral nucleotides. He presented work from the Cameron/Arnold group in developing a quantitative system to study the specificity and efficiency of ExoN exoribonuclease activity and explained how this system was used to investigate the mechanism of proofreading, with the ultimate goal of using this knowledge to antagonize ExoN and prevent its interference with therapeutic efforts. To develop this system, the Cameron/Arnold group expressed and purified components of ExoN (nsp14 and nsp10) for use in activity assays. They found that dsRNA substrates cleaved most efficiently, and that ExoN requires an exposed 3'-RNA terminus to initiate excision. When investigating the efficiency of proofreading, they found that when the enzyme is in excess of substrate, ExoN appears to be highly processive, however under conditions of substrate in excess of enzyme and consistent with its proofreading function, it hydrolyzed only one to two nucleotides in a single binding event. The group found that paired and mispaired ends were excised with similar efficiency, suggesting that access may initiate proofreading instead of mispair recognition. Finally, Jamie presented work identifying modifications to the 3'-RNA terminus that antagonize or completely block ExoN-catalyzed excision, supporting feasibility of future design of ExoN-resistant antiviral nucleotides.

8.5. Efficacy in a SARS-CoV-2 african green monkey model validates a prodrug approach for oral delivery of remdesivir parent nucleoside GS-441524. John Bilello, Ph.D., on behalf of Jared Pitts, Ph.D., Gilead Sciences Inc., Foster City, California, USA

Dr. John Bilello presented work evaluating remdesivir (RDV, VEKLURY®), GS-441524 (the parent nucleoside of RDV), and GS-621763 (a tri-ester prodrug of GS-441524) antiviral efficacy in the African green monkey (AGM) model of SARS-CoV-2. His group found that high exposures of GS-441524 administered intravenously to AGMs reduced infectious virus and viral RNA in bronchioalveolar lavage fluid (BALF) and terminal respiratory tract tissue. However, they also found that GS-441524 must be given in almost 10-fold molar excess to achieve efficacy comparable to that of the 10/5 mg/kg regimen of RDV. John then discussed the oral efficacy of GS-621763 in AGMs. Unlike GS-441524, the prodrug GS-621763 shows high oral bioavailability across multiple species, including nonhuman primates, and can achieve target GS-441524 plasma exposures following oral dosing. Two dosing levels of oral GS-621763 were evaluated in AGMs, with treatment starting ~8 h post infection and continuing daily for 5 days and compared to AGMs treated with matching vehicle controls. Both doses of GS-621763 reduced SARS-CoV-2 RNA and infectious virus in BALF but did not significantly reduce viral RNA in respiratory tract tissues, as seen in mouse and ferret models. Given these data, future work will focus on GS-441524 prodrugs with high oral bioavailability for further development as potential COVID-19 therapies.

8.6. A dual mechanism of action of AT-527 against SARS-CoV-2 polymerase. Ashleigh Shannon, Ph.D., AIX Marseille Université, Marseille, France

Dr. Ashleigh Shannon discussed her group's work on the guanosine analog AT-527 and its active triphosphate form AT-9010. She presented a 2.98 Å cryo-EM structure of the SARS-CoV-2 nsp12/nsp7/nsp8_2-RNA replication-transcription complex, showing AT-9010 bound at three sites of nsp12. One AT-9010 is incorporated at the 3' end of the RNA product strand in the RdRp active site. Its modified ribose group (2'-fluoro, 2'-methyl) prevents correct alignment of the incoming NTP, in this case a second AT-9010, causing immediate chain termination of RNA synthesis. The third AT-9010 is bound in the NiRAN active site (N-terminal domain of nsp12) in a uniquely flipped orientation, with its guanine base unexpectedly occupying a previously overlooked cavity. These binding characteristics allow AT-9010 to outcompete all native nucleotides for binding, inhibiting NiRAN nucleotide transferase activity. Altogether, these data suggest a dual mechanism of action of AT-527 in the targeting of both RdRp and NiRAN activities and support its therapeutic use to treat COVID-19.

8.7. Dual inhibition of SARS-CoV-2 and human rhinovirus with protease inhibitors in clinical development. Jerome Deval, Ph.D., Aligos Therapeutics, San Francisco, California USA

Dr. Jerome Deval discussed his group's work screening inhibitors of the 3-chymotrypsin-like cysteine protease (3CLpro) of SARS-CoV-2. The Aligos program was developed in 2020 in collaboration with CD3 and the Rega Institute at KU Leuven. While many groups use biochemical assays to screen compounds, in particular the FRET assay, Jerome's group chose to screen using a mass spectrometry (MS) approach. In his talk, Jerome shared their experience developing and validating this assay. They found that the MS approach was more sensitive than FRET and offered the advantage of being adaptable to multiplex analyses. Specifically, the group was interested in developing a multiplex assay to simultaneously evaluate inhibitor activity against the rhinovirus HRV3C protease. They used low concentrations of both proteases and validated

the approach with rupintrivir, which is known to selectively inhibit the HRV3C protease. The protease selectivity results were further supported by examining binding structures. The group faced the issue of enzyme titration, which may underestimate compound potency, and performed another round of optimization to further reduce the amount of enzyme to better delineate activity. Jerome finished his talk by briefly mentioning work on resistance selection in collaboration with KU Leuven. In the future, the group is interested in investigating how various 3CLpro inhibitors may induce different resistance mutations and determining the level of cross-resistance that is associated with those mutations.

8.8. In vitro selection and characterization of a SARS-CoV-2 isolate resistant to remdesivir. Dirk Jochmans, Ph.D., KU Leuven – Rega Institute, Leuven, Belgium

Prof. Dirk Jochmans discussed work on GS-441524 and SARS-CoV-2 to investigate whether resistance against remdesivir can be selected *in vitro*. Before his group's work began, an E802D substitution in NSP12 had been described that was related to resistance; however, this mutation also resulted in impaired replication capacity, so its clinical impact remained unknown. Dirk's group performed resistance selection with GS-441524, the parent nucleoside of remdesivir, using a SARS-CoV-2 isolate obtained from a Belgian patient in February 2020; this isolate was closely related to the prototypic Wuhan- Hu-1 2019-nCoV (Wuhan isolate). The virus was serially passaged on Vero-E6 cells, and nucleotide changes in the virus were evaluated. Genetic analysis of the last passage showed mutations in the polymerase gene correlating with amino acid changes S759A + A777S, which were later supported by another group in the field doing comparable studies.

The group went on to investigate resistance to several other compounds, including polymerase inhibitors (GS-441524, molnupiravir) and 3CL-protease inhibitors (PF-00835231, Nirmatrelvir), and observed a significant level of resistance against GS-441524 (EC_{50} increased more than 10-fold [$EC_{50} > 50$ μM]), but no change in EC_{50} for the other inhibitors. The group also looked at the crystal structure, which suggested a unique H-bond between S759 and remdesivir that is lost when the substitution S759A is present. Dirk finished by mentioning his discussions with Bruno Canard regarding the SDD motif and potential for H-bond formation that may affect fidelity of the polymerase and described how these properties could relate to hypersensitivity to molnupiravir. Overall, the work presented required several virus passages (>10) before the compound concentration could be increased, supporting a barrier to resistance development and low possibility of resistance selection during short clinical treatment. Nonetheless, Dirk emphasized the need for actively surveying patients to continue monitoring for putative generation of resistance.

8.9. Oral inhibitors of the SARS-CoV-2 main protease for the treatment of COVID-19. Rhonda Cardin, Ph.D., Pfizer Worldwide Research Development and Medicine, Pearl River, New York, USA

Dr. Rhonda Cardin discussed the discovery of PF-7321332, the first oral SARS-CoV-2 M^{pro} inhibitor to reach clinical development. In just 17 months, this work went from the beginning of a discovery program to EUA approval. Based on the company's experience with SARS-CoV, the Pfizer protease inhibitor investigation program began in response to the COVID-19 pandemic with the goal of identifying an oral protease inhibitor. Their compound screening and quick development efforts utilized measures of virus inhibition (enzymatic inhibition assay and SARS-CoV-2 Vero E6 cell CPE assay), focused on optimizing absorption, distribution, metabolism, and excretion (ADME) and oral bioavailability, and employed rational design techniques. In only 4 months, the project had designated PF-07321332 (nirmatrelvir; the active ingredient in Paxlovid™) as the lead compound possessing high potency across cell lines and broad-spectrum anti-coronavirus activity *in vitro*. In collaboration with Utah State University, the group went on to show the compound's *in vivo* efficacy in a murine model. The compound protected mice from weight loss and reduced viral lung titers. Histology and immunostaining showed dose-dependent reduction in N protein expression. Activity against variants was also assessed. Rhonda completed her talk by presenting data from clinical studies. Phase 1 studies were performed looking at single and multiple ascending doses, which were followed by Phase 2/3 studies. Four Phase 2/3 studies were performed in different populations, including (1) high risk, (2) household contacts, (3) standard risk, and (4) pediatric cohorts; trials in the latter three groups are ongoing with the goal of obtaining full FDA approval for use of Paxlovid in these populations.

8.10. Picomolar covalent reversible inhibitors of CoVs main proteases effectively inhibit SARS-CoV-2 replication: design, synthesis, biological evaluation, and X-ray structural characterization. Vincenzo Summa, Ph.D., Department of Pharmacy, University of Napoles Federico II, Naples, Italy

Prof. Vincenzo Summa's talk focused on the highly conserved main protease (M^{pro}) of SARS-CoV-2, reporting on his group's work on a new series of tripeptides as covalent reversible inhibitors of this protease. Building on the analysis of broad-spectrum inhibitors of coronaviruses, in early 2020, his group began designing SARS-CoV-2 M^{pro} peptide inhibitors characterized by a proline (Pro) at P2 to induce a β-turn confirmation and investigated antiviral activity of these molecules. The most potent compounds showed potency against M^{pro} of both SARS-CoV-2 and MERS-CoV at pM and nM levels and inhibited viral replication without detectable cell toxicity. The group found that the chirality of the Pro substituents affected inhibitory activity. In X-ray studies, they obtained three co-crystal structures of peptidic inhibitors (F2F2020-184, −185, and −197) in complex with SARS-CoV-2 M^{pro} and revealed that the P2 Pro substituents with favored chirality fit within the S2 pocket. Molecular modeling studies aimed at rationalizing the structure–activity relationship of the designed inhibitors as well as the inhibitory activity against MERS M^{pro} are ongoing.

9. Session 6: hepatitis and herpes viruses

9.1. Clinical update on viral RNA targeting agents for chronic hepatitis B. Fabien Zoulim, M.D., Ph.D., INSERM, Lyon, France

Persistent high antigen load is a major factor driving the exhaustion of antiviral adaptive immunity in patients with chronic HBV infection. Currently, nucleo(s/t)ide (Nuc) administration induces viremia suppression but not viral antigen (mainly HBsAg) reduction. One concept to achieve functional cure of HBV infection, which is defined by the sustained loss of HBsAg in serum, is to decrease circulating HBsAg levels and liver cccDNA levels with the hope to restore antiviral immunity. **Dr. Fabien Zoulim** provided an overview of HBV RNA targeting agents aiming at reducing antigen burden, which represent a promising class of drugs that could be combined with direct immunotherapies to achieve a synergistic effect. Development of transcription inhibitors and mRNA destabilizers has been hampered by toxicities inherent with targeting a host pathway. In contrast, both the Gal-NAc conjugated siRNAs and the naked ASOs appear to be safe and well tolerated when administered subcutaneously.

The first demonstration of HBsAg loss in patients with an siRNA was obtained with ARC-20 (Arrowhead), but this siRNA was discontinued because it did not have any effect against mRNA transcripts from integrated DNA in HBeAg(−) patients, due to its target gene location. Further optimization with a new siRNA sequence (ARO-HBV1001) resulted in potent activity against HBeAg(−) patients. All siRNAs in Phase II clinical development (JNJ-3989; VIR-2218; RG-6346; AB-729) achieve on-treatment HBsAg responses, which is durable for several months after the final dose. Unfortunately, the results also showed a

plateauing of HBsAg, and no patients achieved HBsAg loss. Interestingly, the first study combining an siRNA (VIR-2218) with pegylated IFN showed a synergistic effect on HBsAg reduction to undetectable levels in some patients, which was associated with ALT elevations. In contrast, combination of JNJ-3989 siRNA with a capsid inhibitor (JNJ-6379) and Nuc resulted in lower level of sAg reduction compared with the siRNA + Nuc without capsid inhibitor, suggesting antagonism between the two new modalities (REEF-1 Study). The most advanced ASO is GSK-836. Biweekly GSK-836 300 mg dosing achieved 3–4 log reductions in HBsAg within 28 days, associated with ALT elevation that may suggest some level of immune restoration. Whether this will lead to sustained HBsAg loss and functional cure is currently investigated in a phase II trial. Results in mouse models showed that siRNA knockdown of HBV transcripts followed by therapeutic vaccination allowed viral clearance. Ongoing phase Ib/II clinical trials will determine if functional cure can be achieved with this type of combination therapy.

9.2. Discovery and development of HBV core inhibitors for the treatment of chronic hepatitis B infection. William Delaney, Ph.D., Assembly Bio, South San Francisco, California, USA

With almost 300 million HBV-infected individuals worldwide, chronic hepatitis B remains a major global public health problem. Nucleoside analogs are a life-long therapy with very low cure rate. **Dr. Bill Delaney** provided an overview of the research done on HBV core inhibitors. Core inhibitors are a new class of antivirals that can deepen viral suppression and have the potential to improve cure rates in patients with chronic HBV infection when used in combination regimens. Core inhibitors interact allosterically with HBV core protein and modulate assembly and stability of nucleocapsids. Core protein is critical both early and late in HBV replication, and thus core inhibitors have multiple mechanisms of action (MOAs) including: (1) inhibition of pgRNA encapsidation (preventing assembly and release of new viral particles); (2) blocking intracellular amplification of cccDNA; and (3) disrupting incoming capsids, which prevents *de novo* cccDNA formation. It is generally considered that potent activity against all three MOAs is likely to be important for maximal viral suppression. Bill also summarized the past and current clinical research landscape for core inhibitors. Multiple and chemically diverse core inhibitors are progressing through preclinical, phase 1, and phase 2 development. Phase 1 studies have confirmed that core inhibitors such as Vebicorvir (VBR) have potent antiviral activity including multi-log suppression of both serum HBV DNA and RNA when used in monotherapy, with the latter effect not observed with nucleos(t)ide reverse transcriptase inhibitors (NrtIs). Phase 2 combination studies have demonstrated that addition of VBR to the NrtIs entecavir (ETV) for 24 weeks deepens suppression of both HBV DNA and RNA compared to ETV alone. At the end of treatment, 83% and 59% of HBeAg + patients receiving the combination treatment had undetectable levels of viral DNA and RNA, respectively. This compares to 29% and 18% of patients receiving ETV alone.

Importantly, there has been no evidence for the emergence of core inhibitor resistance in patients compliant with combination therapy. However, all patients meeting stopping criteria and taken off treatment experienced rebounds in HBV DNA after discontinuation of combination treatment, indicating that they had not been cured. Relapse indicates that the reservoir of cccDNA had not been depleted, possibly because VBR is not potent enough to block cccDNA replenishing (2nd mechanism). Current studies are investigating the curative potential of triple combination regimens consisting of core inhibitors, NrtIs, and various third agents (siRNA, interferon, TLR7 agonist). As these agents are further evaluated, next-generation core inhibitors with significantly greater potency, particularly against cccDNA formation, are in early clinical development.

9.3. Explore viral infection to probe roles of protein deamidation. Pinghui Feng, Ph.D. University of Southern California, Los Angeles, California, USA

Prof. Pinghui Feng began his presentation by describing his laboratory's experience working on human Kaposi's sarcoma-associated herpesvirus (KSHV) and herpes simplex virus 1 (HSV-1), and how they expanded this knowledge to include a focus on cancer biology and SARS-CoV-2. Pinghui started his lab studying how these viruses interacted with the innate immune signaling pathways during infection, showing that, despite an active immune response, herpesviruses establish persistent infection in diverse tissues. By studying these viral immune evasion mechanisms, he discovered that herpesviruses could deploy pseudo-enzymes to target key pattern recognition receptors for deamidation, for example hijacking the RIG-I pathway to block antiviral cytokine production. He discussed how these studies implicated cellular glutamine amidotransferases (GATs) in deamidating proteins, and then went onto to describe his investigations into the roles these enzymes play in regulating innate immune pathways. In one such example, Pinghui described how cancer cells can hijack RelA, an innate immune defense component involved in NF-κB activation, via CAD-mediated deamidation resulting in metabolic reprogramming of RelA to instead promoting aerobic glycolysis and tumor cell proliferation. He described his efforts investigating the potential roles of protein deamidation in SARS-CoV-2 infections, during which they identified several SARS-CoV-2 proteins that interacted with CAD, including Nsp9. He presented data showing that Nsp9 activated CAD via recruitment of S6K1, and that inhibition of S6K1 abolished Nsp9-mediated CAD activation and subsequent downstream events including *de novo* pyrimidine synthesis and suppression of NF-κB activation. Pinghui concluded his presentation by showing data on a glutamine analog small-molecule inhibitor, 2-TCPA. He presented *in vitro* data showing that 2-TCPA treatment elevated the inflammatory response, inhibited *de novo* nucleotide synthesis, and was able to inhibit SARS-CoV-2 replication in Caco-2 cells with an EC_{50} of 2.36 μM. In mice, 2-TCPA treatment caused upregulation of several antiviral genes and was able to reduce viral titers by over 3 logs.

9.4. LAVR-289, a new broadly active acyclonucleoside phosphonate prodrug, is highly effective in the SCID-Hu mouse model of varicella zoster virus replication. Jennifer Moffat, Ph.D., SUNY Upstate Medical University, Syracuse, New York, USA

Prof. Jennifer Moffat discussed her group's work on evaluating LAVR-289, a new acyclic nucleobase phosphonate (ANP), in both cells and a mouse model. In ARPE-19 cells, LAVR-289 was highly effective against wild-type varicella zoster virus (VZV), demonstrating greater efficacy than cidofovir and acyclovir; it was also effective against acyclovir-resistant VZV. The group utilized a human skin tissue model, used either ex vivo or engrafted subcutaneously onto immunodeficient mice and subsequently inoculated using a reporter VZV. When LAVR-289 was added to the medium underlying the ex vivo skin tissue culture, its effects were comparable to those of cidofovir; future studies will examine topical application to determine if this treatment approach will enhance efficacy of LAVR-289 treatment. In mice, LAVR-289 prevented VZV spread similarly to cidofovir. In addition, while cidofovir-treated mice lost weight, no clinical signs were seen in mice treated with LAVR-289, suggesting that the novel compound may be better tolerated than other treatment options. Subsequent studies in mice tested delayed initiation of treatment and using a lower dose; LAVR-289 remained highly effective even at one-fourth of the initially evaluated dose and starting 3 days after infection, which translates well to an achievable clinical intervention window.

9.5. Characterization of the N-hydroxypyridinediones (HPD) and the N-(HNO) as HBV RNase H inhibitors. Molly Woodson, M.S., Saint Louis University School of Medicine Department of Molecular Microbiology and Immunology, St. Louis, Missouri, USA

Molly Woodson (Ph.D. candidate) presented their work on RNase H inhibitors against HBV. She started by covering the basics of HBV, an hepatotropic hepadnaviridae with a 3.2 Kb partially dsNA genome. It is estimated that up to 300M people are infected with HBV, and about 880,000 die per year due to HBV complications. The current treatments are based on nucleosides and IFN alpha. They fail to cure the infection requiring lifelong treatments, which is a very high barrier in the parts of the world where treatment is the most necessary. She discussed that it is commonly accepted that combination therapy is required. RNase H plays a critical role in viral replication and its H inhibition inhibits the synthesis of positive strand DNA as the DNA-RNA duplex is not resolved. Currently, there are not drugs that inhibit HBV RNase H. N-hydroxypyridinediones (HPD) were identified as a family of hits against HBV RNase H. The oxygen trident is required, and oxime bonds increase potency. They evaluated ortho, meta, and para substitutions on the benzene ring. The four best compounds have di-fluor (orthro and para), or single nitrile (para), methyl (meta), or fluor (meta) substitutions. In summary, the best compounds have electron withdrawing groups at the meta and para positions of the benzene ring.

The N-hydroxynapthyridinones (HNO) are another chemotype identified as HBV RNase H inhibitors and HBV inhibitors. HNO are moderately toxic, though, and thus modifications to eliminate hepatotoxicity were introduced. The EC_{50} was kept in the low micromolar range and cytotoxicity was significantly decreased, yielding compound 1562 with an SI about 45. They next evaluated the effects of pH on solubility focusing on pH 7.4, 6.5 or 5, representing the different pH along the GI tract. By PAMPA (parallel artificial membrane permeability assay) analyses, the compounds have higher passive permeability at pH5 than 7.4. During the question-and-answer period, she stated that there is strong interest in moving into PK studies in animal models, with no pre-established criteria to proceed. The issue of coinfection with HIV was discussed as was the potential activity against HIV RT, as the HPD were originally identified as HIV RNase H inhibitors. In brief, Molly's talk discussed two families of HBV RNase H inhibitors and their potential toward developing new anti HBV antivirals.

9.6. Resistance analysis in a phase 2 clinical trial with the helicase-primase inhibitor pritelivir in immunocompromised adults with acyclovir resistant herpes simplex virus (HSV) infection. Alexander Birkmann, Ph. D., AiCuris Anti-infective Cures AG, Wuppertal, Germany

Dr. Alexander Birkmann presented the results of the phase II PRIOH-1 clinical trial, performed as a collaboration with Keith R Jerome (University of Washington), which is now continuing as a Phase III. Pritelivir targets the helicase primase complex, which is a novel and unique target. It needs no activation by TK and thus is active against TK-resistant strains, but resistance can be selected for in culture and maps to single mutations in UL5 helicase or UL52 primase.

The PRIOH-1 is a randomized, open label, multi center, comparator-controlled clinical trial in immunocompromised subjects. Part A included patients with infections resistant only to ACV. Pritelivir treatment started with a 400 mg loading dose followed by 100 m po QD, and the comparator was forscarnet iv 40 mg/kg TID or 60 mg/kg BID; randomization was 2:1. Part B included patients infected with mutants resistant to both ACV and foscarnet or with ACV resistant virus and foscarnet intolerant, so there was no comparator. Treatment was for up to 28 days or until lesion healed (whichever occurred first) and the follow up was for another 28 days, with visits every other day starting on day 3 during the treatment day and on day 56 after starting treatment. If the lesions had not healed on the final visit, another visit was included either when the patient reported healing or at maximum 56 days after treatment initiation. The Inclusion criteria was confirmed ACV resistance. Herpes simplex virus (HSV)-1 DNA was quantitated by PCR during treatment, and resistance in new lesions at end of trial was tested. Resistance to ACV was evaluated by genotypic screening follow up by phenotypic screening if the genetic test was inconclusive. Viral shedding during treatment was evaluated by PCR and the genotype was tested again at the end of treatment for resistance to pritelivir, ACV or foscarnet if lesions were visible. In total, one patient developed resistance to pritelivir and one to foscarnet in part A of the study, and none in part B, for a total 4.3% resistance to pritelivir and 14.23% to foscarnet in this small sample size.

Both resistant mutants were HSV-2, and both were previously known mutations (UL5 mutated in K355T for pritelivir and U30 polymerase E98K for foscarnet), conferring 12.3 and 2.3-fold resistance, respectively. Resistance to pritelivir developed in a male patient with HIV and genital herpes which did not heal during treatment, although viral shedding decreased by about 200-fold and the lesion area decreased by also close to 200-fold. Plasma concentrations of pritelivir were in normal ranges and no unusual co-medication was administered, but the immune status of the patient is unknown. The mutation had been identified before in HSV-1. By NGS, they could observe the emergence of the mutant between days 3 and 5 to then become the dominant population, eventually replacing the wild type of virus by day 11.

This was the first reported resistance to pritelivir in the clinics. During the question-and-answer period it was speculated whether the pre-existing ACV resistance may be lost during treatment, and that the patients under pritelivir had far fewer side effects than those in foscarnet, to the point that they are having trouble recruiting new patients because all want to go to the pritelivir arm and not to the foscarnet one.

10. Late breaking oral presentation

10.1. Intermittent therapy with helicase-primase drug candidate IM-250 reduces reactivation competency of latent Neural herpes simplex virus infections. Gerald Kleymann, Ph.D., Innovative Molecules GmbH, Munich, Bavaria, Germany

Herpes simplex virus (HSV) causes widespread genital and oropharyngeal disease; less often, it also causes encephalitis, sight impairing keratitis, and neonatal or disseminated herpes. HSV establishes lifelong latent infections in neurons supporting viral reactivation and recurrent disease. Standard of care treatments with nucleoside analogs include valacyclovir, acyclovir or famciclovir, with foscarnet being used as salvage therapy despite being poorly tolerated. More effective therapies are needed for CNS infections, resistant viruses, and, ideally, for silencing recurrences after cessation of treatment. **Dr. Gerald Kleymann** presented an update on the HSV helicase-primase inhibitor IM-250, with potent in-vitro and in-vivo activity against HSV including nucleoside-resistant mutants due to its different mechanism of action. IM-250 is about 100-fold more potent than acyclovir in cell culture. IM-250 has a long half-life, slow clearance, and good bioavailability, resulting in a unique high CNS target tissue exposure in rodents and dogs.

Gerald also showed that early therapy of genital herpes in guinea pigs significantly reduces primary disease, ganglionic viral load, and recurrent disease. Importantly, IM-250 reduced recurrences for a time after cessation of treatment, reduction which was not observed with the standard of care valaciclovir. To further investigate the ability of IM-250 to influence reactivation after latency has been established and after cessation of treatment, viral reactivation at the single neuron level was examined in the mouse ocular model. Four cycles of intermittent treatment (IT, 1-week IM-250 or placebo in food followed by 2-weeks normal chow) were initiated beginning 45 days after HSV-1 ocular infection. Viral reactivation induced 15 days after final IT resulted in 2-fold fewer positive ganglia (7/24 and 14/24), and a 3.6-fold reduction in total neurons undergoing reactivation in IM-250 vs placebo treated mice ($p =$

0.002). Confirmed in a smaller study, these findings support the hypothesis that intermittent treatment with IM-250 alters the pool of latent reactivatable virus, a novel and a major advance for HSV therapies. Clinical trials to confirm these murine (HSV-1) and guinea pig (HSV-2) findings are planned.

10.2. A bifunctional immune modulator exhibits potent antiviral activity in HBV infection models. Antoine Alam, Ph.D., Evotec, Lyon, France

With almost 300 million HBV-infected individuals worldwide and >800,000 deaths annually due to hepatocellular carcinoma, chronic hepatitis B (CHB) remains a major global public health problem. There are currently only two modalities available for treatment: Type-I interferons (IFN–I) and nucleo(s/t)ide analogs (Nucs). IFNs and Nucs have shown limited ability to cure patients with CHB, thus more effective treatments are needed to reach a cure in a significant proportion of patients. Cluster of differentiation 40 (CD40) is a TNF receptor located on antigen-presenting cells and recently found to enhance IFN response to HBV infection. Antoine showed that simultaneous stimulation of CD40 and IFN-I pathways *in vitro* and *in vivo* with HBV infection models increased the antiviral efficacy compared to IFN-I alone, without further inducing inflammatory cytokines. The combination of IFN-I and CD40L, on primary human hepatocytes (PHH) and in AAV/HBV-infected mice, showed a significant increase in anti-HBV activity when compared to either CD40L or IFN-α alone. To maximize dual target engagement of IFN-I and CD40L with appropriate exposures in the liver, the IFN-I molecule was fused to an anti-CD40 agonistic mAb. This yielded a bifunctional molecule active on both CD40 and IFNR reporter cells capable of delivering both activities to HBV infected hepatocytes *in vivo*. Interestingly, the fusion molecule is more active than the parental antibody in the HEK-CD40 reporter assay. At picomolar concentrations, the fusion molecule is also able to reduce viral products from HBV-infected PHH treated for a period of 4 days after infection without cytotoxicity. Stimulation of CXCL10 release and anti-HBV activity was maintained in infected PHH treated for 1 day followed by washout period of 3 days. These results demonstrate the feasibility of combined stimulation of CD40 and IFN-I pathways with a single bifunctional molecule to achieve potent activity against HBV.

10.3. The nucleoside analog antiviral CMX521 inhibits SARS-CoV-2 in human airway epithelial cell cultures and exhibits prophylactic and therapeutic efficacy against respiratory disease in a mouse model of SARS-CoV-2 infection. Randall Lanier, Ph.D., Chimerix, Durham, North Carolina, USA

Dr. Randall Lanier started his talk by highlighting that recently there have been coronavirus outbreaks every 6–8 years, and thus we should be ready for the potential for future outbreaks with similar viruses. We therefore still need treatments and prophylactic agents. His talk focused on CMX521, a ribonucleoside triphosphate inhibitor of norovirus RdRP. The IND activities performed for another indication (norovirus) showed good oral tolerability in GLP studies with rats and dogs, and single dose trials in humans. Completed Phase I clinical trials demonstrated an excellent safety profile, and no significant activity against human polymerases, a common source of toxicity for nucleoside analogs. *In vitro*, CMX521 has low μM activity across coronaviruses in human airway epithelial cells, but poor activity in a monkey kidney cell line (Vero). Oral or intravenous delivery also showed suboptimal delivery of the drug to the lungs. Changing the cell type and animal model took the project forward. As the coronavirus RdRP active site is highly conserved it was tested against mouse and human coronaviruses and showed similar potency. CMX521 was tested in the mouse adapted SARS-CoV-2 MA10 model using aerosol delivery by nebulization in PBS at 5 mg per chamber with one mouse per chamber-the actual intake by the animal is expected to be much lower. Progression of disease is very fast in this model, and Randall proposed that 1 day in mouse is equivalent to about 5–7 days in humans. Clinical and virological parameters were evaluated. Viral lung titer decreased by about 1.4–3.5 logs if used from 5 h before up to 16 h after infection, and lung pathology was ameliorated. The clinical scoring reflected the pathological findings, and treatment protected against weight loss. These studies support further advancement toward clinical trials. Randall anticipated that the bridging tox studies would be the most time demanding step required, and the potential is there to initiate clinical POC within 1 year as a nebulization treatment. Randall demonstrated the potential at home use of a handheld nebulizer purchased from a popular online retailer. In summary, Randall's talk described the progression of CMX521 as a potential antiviral against SARS-CoV-2.

*10.4. Structural and mechanistic characterization of non-neutralizing antibodies targeting Crimean-Congo hemorrhagic fever, ian durie, Ph.D. Candidate, University of Georgia, Athens, Georgia, USA**

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

11. Pechakucha competition

Finalists: Daniel Bradley, Winston Chiu, Li-Hsin Li, Chloe Monet-Murrell, Katerina Radilova, Joy Thames, from USA, Belgium, and the Czech Republic. First Prize ($250) Joy Thames, University of Maryland, Baltimore County, USA. Second Prize ($100) Winston Chiu, KU Leuven, Belgium. Third Prize ($75) Daniel Bradley, Saint Louis University, Saint Louis, Missouri, USA.

12. Poster awards

This year's ICAR poster award competition in Seattle had two venues for presentations (and for awards) – in-person and virtual. The international team of judges included Antoine Alam, John Bilello, Andrea Brancale, Jinhong Chang, Leen Delang, Yanming Du, Cybele Garcia, Brian Gentry (chair), Brett Hurst, Chris Meier, Jennifer Moffat, Joana Duarte Da Rocha Pereira, Luis Schang, Kathie Seley-Radtke, Jessica Spengler, Pei Yong Shi, Enzo Tramontano, Subhash Vasudevan, and Zhengqiang Wang. Thirty-six posters registered to be judged in-person and twenty-one posters registered to be judged virtually in the three categories (undergraduate/graduate student, postdoctoral researcher, and young investigator).

The winner of the in-person Young Investigator category was Poster #112 "The phenanthroindolizidine (-)-13aR-6-O-desmethyl-antofine inhibits Zika virus replication in human cells" by Chaker El Kalamouni who received $1000 and a publication fee waiver for one publication

within the next year to Antiviral Chemistry and Chemotherapy. The winner of the virtual Young Investigator category was Poster #157V "Oral Heat Shock Protein 90 (Hsp90) Inhibitor SNX-5422 Attenuates SARS-CoV-2 Replication And Dampens Inflammation In Airway Cells" by Ria Goswami who received $750.

The winner of the in-person postdoctoral category was Poster #114 "A Yellow Fever Virus NS4B Inhibitor Enhances the Activation of Multiple RNA Sensing Pathways and Induces Pre-mature Death of Infected Cells" by Fuxuan Wang who received $1000. The winner of the virtual postdoctoral category was Poster #163V "Exploring the morphology of the host cell to open new possibilities in drug repurposing" by Marianna Tampere who received $750. The runner-up in the virtual postdoctoral category was Poster #102V "Designing and Evaluating Neutralizing and Fusion Inhibitory Antiviral Peptides to a Tick-Transmitted Hemorrhagic Fever Virus" by Sergio Rodriguez who received $250.

The winner of the in-person Graduate Student category was Poster #200 "Structural basis of nucleotide recognition by the SARS-CoV-2 RNA dependent RNA polymerase" by Brandon Malone who received $1000 and the Journal of General Virology publication waiver award. The three runners-up for the in-person Graduate Student category were poster #141 "Hits on the Virus RGB Palette" by Li-Hsin Li, poster # 232 "Antiviral Activity of Peptide A-3302-B Isolated From a Marine Bacterium Micromonospora sp. Against Herpes Simplex Virus Type 2" by Irene Arduino, and poster #261 "Unravelling the anti-influenza effect of flavonoids: Experimental validation of luteolin and its congeners as potent influenza endonuclease inhibitors" by Katerina Radilova (each runner-up received $500). The winner of the virtual Graduate Student category was Poster #162V "Development of N-Substituted Furopyrroles as EBOV and MARV Anti-filoviral Glycoprotein Inhibitors" by Destiny Durante who received $750.

13. Chu Family Foundation awards

Each awardee ($3000): Laura Taracón Díez (postdoctoral fellow, Laboratory of Molecular ImmunoBiology, Hospital Gregorio Marañón, Madrid, Spain), Ana Lucia Rosales Rosas (graduate student, Rega Institute for Medical Research, KU Leuven, Belgium), Ashleigh Shannon (postdoctoral fellow, AFMB, Aix-Marseille Université, Marseille, France).

14. Concluding remarks

The 35th ICAR was a much-welcomed and very much enjoyed return to an in-person annual meeting. All sessions and special events were well attended and reinforced the importance of collaborations, as well as the growing and evolving field of antiviral research. Through ICAR and other annual outreach efforts, ISAR continues to promote and foster the antiviral research community. The 36th ICAR, which will be held in Lyon, France, March 13–17, 2023, aims to carry on this mission and follow in the success of this year's meeting.

Note

Presentations that were reported using the speaker-submitted abstract are indicated with an asterisk (*).

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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