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Meeting report: 34th international conference on antiviral research

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Abstract

As a result of the multiple gathering and travels restrictions during the SARS-CoV-2 pandemic, the annual meeting of the International Society for Antiviral Research (ISAR), the International Conference on Antiviral Research (ICAR), could not be held in person in 2021. Nonetheless, ISAR successfully organized a remote conference, retaining the most critical aspects of all ICARs, a collegiate gathering of researchers in academia, industry, government and non-governmental institutions working to develop, identify, and evaluate effective antiviral therapy for the benefit of all human beings. This article highlights the 2021 remote meeting, which presented the advances and objectives of antiviral and vaccine discovery, research, and development. The meeting resulted in a dynamic and effective exchange of ideas and information, positively impacting the prompt progress towards new and effective prophylaxis and therapeutics.

Keywords

Virus, antiviral, therapeutics, vaccines, ICAR, ISAR meeting

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Introduction

As a result of the SARS-CoV-2/COVID-19 pandemic, ICAR 2021 was held virtually from Monday, March 22, through Friday, March 26, 2021, using the OnAIR virtual event platform. ISAR was fully committed to make this virtual meeting an interactive experience by preserving as many of the components of past in-person ICAR meetings as possible. Of course, the attendees could not enjoy casual (and not so casual) conversations over coffee breaks, cheese and wine poster sessions, lunches, dinners, or banquets. Nonetheless, they were given ample access to all speakers and to each other, and the sessions were marked by lively interactions between attendees, presenters, and panel members. The virtual platform used, and the decision of having the sessions pre-recorded and available for pre- (and post-) viewing, were conducive to many productive interactions. The interactivity was particularly highlighted during the speed dating, women in science, poster awards, and PechaKucha competition activities. This last activity was as lively and enjoyable as usual, although some presenters came to realize the challenges of having complex videos in their slides during remote presentations. As ISAR is an international society, the interactive live sessions were organized around two prime-time periods to accommodate speakers and attendees from around the world, held 11:00 am through 1:00 pm and 8:00 pm through 10:00pm EST In addition, attendees were able to listen to the talks, which were all recorded, at their leisure.

Similar to in-person meetings of previous years, the virtual ICAR2021 program was outstanding, including topical keynote lectures on SARS-CoV-2 vaccines, antivirals, and a variety of models, as well as on pandemic preparedness. As other viruses are not going away, the problems caused by them still demand new antivirals. The sessions on influenza, respiratory syncytial virus (RSV), herpes, hepatitis, retroviruses, and arboviruses were complemented with sessions on therapeutics and innovations. Altogether, the program provided an excellent update on the many advances in the field, from understanding the pathogenesis of viral diseases to optimizing new chemical entities targeting them.

Below are summaries of the presentations prepared by the session Chairs and other contributors to provide an overview of the excellence of ICAR2021.

Session 1: influenza and respiratory syncytial virus

Treating RSV infection. Louis Bont, M.D., Ph.D., University Medical Center Utrecht, Utrecht, Netherlands

Louis Bont discussed the incidence and impact of RSV in the Netherlands and globally, noting severe disease in both pediatric and elderly patients. While currently no treatment is approved for RSV bronchiolitis, **Louis** presented several considerations that may improve intervention options: timing of treatment, simultaneous use of immunomodulators, and the promise of new antibodies that are both efficacious and stable over extended time periods.

The F protein of RSV, a key target for antivirals, has two conformational variants: a functional pre-fusion confirmation and an inert post-fusion confirmation. Epitopes differ between conformations; antibodies directed at the pre-fusion epitopes are highly neutralizing, while those directed against post-fusion epitopes are not. Numerous RSV antivirals are under development, including antibodies (mostly against F), fusion inhibitors (also targeting F), and nucleoside analogues targeting intracellular RSV. A healthy human challenge model for RSV is used to evaluate antiviral-mediated replication inhibition in low-risk populations. However, high efficacy in vitro often does not translate to improved clinical outcomes. For example, presatovir, a fusion inhibitor, does not decrease viral loads or improve the clinical response in patients treated 4–5 days after symptom onset. Similarly, while inhaled ALX-0171, a formulation of antibodies against RSV F, dose-dependently decrease RSV replication in airways of infected infants treated 3 days after onset of symptoms, it did not lead to a positive clinical response. These studies and data from other respiratory virus antivirals suggest that clinical development of RSV treatments should focus on initiating treatment earlier, within 24 h of symptom onset.

Is stopping viral replication the way forward, or should other contributors to viral pathogenesis also be modulated? Louis discussed how immunopathogenesis may explain challenges in developing antivirals, and suggested immune system suppression in conjunction with antiviral treatment. In particular, high levels of neutrophils are seen in the airways of RSV-infected children; these are phagocytosing cells that degranulate and form neutrophil extracellular traps (NETs). Checkpoint treatment can decrease formation of NETosis and may work in conjunction with antivirals to improve outcome.

Finally, Louis discussed vaccines and immunoprophylaxis. He highlighted next-generation monoclonal antibodies (mAb) with demonstrated potency and notably long half-lives (MEDI8897), which allow a single prophylactic injection to effectively prevent severe RSV in the first year of life. However, he cautioned that mAb-resistant RSV mutants have been selected in vitro to these newly highly promising treatments, suggesting that though they are not yet seen in nature, they may occur, as has happened in clinical trials with other candidate mAb.

The polymerase complex of the non-segmented negative strand RNA viruses: differences in initiation mechanisms. Rachel Fearns, Ph.D., Boston University School of Medicine, Boston, Massachusetts, USA

Rachel Fearns centered her lecture in the context of the work of her lab, which studies RNA-dependent RNA

polymerases (RdRP) of non-segmented, negative-strand RNA viruses (nsNSVs), particularly that of RSV. In RSV, as in other nsNSVs, the genome is used both for transcription, providing capped and polyadenylated mRNAs, and for replication, which produces the encapsidated antigenome RNA. Her group has found that RSV polymerase initiates RNA synthesis from two different sites: position 1U in the promoter region begins RNA replication, while position 3C begins transcription, the latter being dominant. Rachel's lab is studying in detail how start site usage is controlled using cell-based minigenome and cell-free biochemical assays. The evidence to date points to relative concentrations of NTP, particularly ATP and GTP, and promoter sequences as the main determining factors for start site usage. The group has also addressed whether similar control strategies are used by other nsNSVs. So far, Rachel's group has found that human metapneumovirus (HMPV), which, like RSV, belongs to the family *Pneumoviridae*, has a similar promoter sequence and probably uses a similar mechanism as RSV. However, viruses in the families *Paramyxoviridae* or *Rhabdoviridae* (e.g., vesicular stomatitis virus [VSV]) appear to have a single initiation site at 1U.

These data from the biochemical assays examining RSV initiation mechanisms were further analysed at the structural level. During the formation of the initiation complex, the priming loop contains an aromatic or proline residue that interacts through stacking interactions with the incoming NTP. The VSV priming loop extends into the active site, whereas the putative RSV priming loop does not. Thus, Rachel's group performed mutational analysis of the aromatic and proline residues in this presumed RSV priming loop. The data indicated that Pro1261 indeed stabilizes the initiation complex and may also function as a priming residue. When mutated to Ala, the L_{P1261A} defect was overcome with high concentrations of NTPs or by changing the divalent cation from Mg^{2+} to Mn^{2+}. The more open structure of RSV, in which the priming loop does not project into the active site, might be required for the initiation complex to be able to start from two different positions (1U or 3C). Dissecting the similarities and differences among the polymerases of nsNSVs will be very helpful for the development of broad antiviral strategies.

Now that we have the world's attention, posed the question: are we prepared for the next pandemic? Ron Fouchier, Ph.D., Erasmus Mc Rotterdam, Dept Viroscience, Rotterdam, Netherlands

In his talk, **Ron Fouchier** emphasized the zoonotic nature of many respiratory viruses and the importance of animal hosts in pandemics, and discussed vaccination of animals and limiting exposure to potentially infectious hosts as approaches to mitigate human disease. He spoke about a generalized lack of global preparedness for pandemics and a need for pro-active programs, preferably intervening when changes occur in animal populations, or at least shortly after disease spreads to humans. Ideally, spillover into domestic animals and humans could be prevented, but if it did occur, broadly acting antivirals would be available.

Influenza is an important example of a zoonotic agent with pandemic potential. Influenza pandemics are frequent and range in severity, and mortality is high in interpandemic years. A single zoonotic event becomes a threat when it acquires the ability for human-to-human transmission. Ron presented ongoing research on influenza to identify high-risk viruses among a diverse virus family and to design broadly active vaccine candidates against these viruses. When investigating whether bird influenza virus could acquire the ability to transmit between mammalian models through droplets and aerosols, Ron's group identified three traits required to confer this phenotype: (1) a switch in receptor specificity; (2) pH stabilization of the HA protein; and (3) polymerase adaptation that allows replication at lower temperatures in mammalian upper airways. This knowledge can now be applied in "smart surveillance" to permit early intervention, stopping outbreaks in domestic species and preventing putative pandemics when changes detected in surveyed virus samples indicate increased pandemic potential. Ron finished by discussing pre-pandemic vaccine design and development of a universal H5 vaccine candidate. He noted potential added value in using biological response modifiers in treatment regimens and a desire to develop improved options for use in pandemic scenarios.

In summary, Ron expressed the need for better preparedness for future pandemics via the development and use of diagnostics and surveillance networks, basic research of infectious disease pandemic threats, preventive actions (e.g., at the infected host-human interface), novel vaccine and antiviral development, and availability of generic drugs that may prevent co-morbidities and improve speed of recovery.

The flavonoid cyanidin shows antiviral and immunomodulatory properties against RSV, in vitro and in vivo. Carlos Bueno, Ph.D., Departamento de Química Biológica-IQUIBICEN, Universidad de Buenos Aires, Argentina

Carlos Bueno presented the fourth talk of the session. He started by highlighting that RSV is able to interfere with the immune system and the host response. Interfering with the immune response could thus be exploited for therapeutic strategies against RSV. Carlos' presentation centered on cyanidin, a flavonoid present in red berries and other fruits. The anti-inflammatory effects of this natural product have been described, and in his presentation,

Carlos analyzed its antiviral activity and immunomodulatory properties against RSV in vitro and in vivo. In particular, he showed that cyanidin modulated cytokine production in RSV-infected epithelial cells and TLR-stimulated epithelial cells. Treatment with cyanidin in a murine model of RSV improved the course of the acute disease, particularly evidenced by reduced RSV titers in the lungs and attenuated airway inflammation. The presented data support considering cyanidin as a potential therapeutic alternative against RSV infection.

Antagonism by a baloxavir and oseltamivir drug combination against baloxavir-resistant, but not against oseltamivir-resistant influenza a virus infections in mice. Scott Gibson, B.S., Institute for Antiviral Research, Utah State University, Logan, Utah, USA

The presentation by **Scott Gibson** illustrated different outcomes of drug combinations in treating influenza depending on the pre-existence of drug-resistant mutations. Scott used two drugs with different mechanisms of action: baloxavir marboxil, an inhibitor of influenza cap-dependent endonuclease, and oseltamivir, a well-established neuraminidase inhibitor. A baloxavir marboxil-resistant influenza A/California/04/2009 (H1N1pdm) virus, selected by passaging the virus in increasing concentrations of baloxavir marboxil, and an oseltamivir-resistant clinical isolate influenza, A/Hong Kong/2369/2009 (H1N1pdm), were used to challenge BALB/c mice. Combination therapy was less protective than oseltamivir monotherapy against the baloxavir-resistant virus, suggesting strong antagonism of the oseltamivir-baloxavir combination against a baloxavir-resistant virus. Conversely, combination therapy provided higher protection against oseltamivir-resistant influenza virus compared to oseltamivir monotherapy. Baloxavir marboxil alone or in combination with oseltamivir showed a potent antiviral effect against an oseltamivir-resistant, highly pathogenic avian influenza virus, A/Taiwan/1/2017 H7N9.

A single-dose live-attenuated YF17D-vectored SARS-Cov-2 vaccine candidate, Lorena Sanchez-Felipe, Ph.D., KU Leuven, Rega Institute, Leuven, Belgium

Lorena Sanchez-Felipe presented the results of a large collaboration between multiple institutions testing the efficacy of a SARS-CoV-2 vaccine candidate based on the live-attenuated YF17D vaccine. Three constructs containing the wild-type protein expressing S1 and S2 and a cleavage mutant version that only expresses S9 or one that expresses only S1, all inserted between E and NS1-5, were produced. All constructs expressed the recombinant genes and all produced small plaque phenotype, demonstrating attenuation. The vaccines were tested in a hamster challenge model; the S1-expressing construct was not highly immunogenic or protective, but the other two were, stimulating strong antibody responses and protecting against a high dose challenge (2×10^5 PFU/animal). Large reductions in viral infectivity and RNA levels in the lungs and reduced pathology were noted at necropsy. Cell responses were evaluated in a mouse model and, interestingly, the S1-expressing construct, which was not efficacious in hamsters, induced the highest levels of CD4 and CD8 cells expressing interferon (IFN)-γ and tumor necrosis factor (TNF)-α. As expected, the vaccine also protects mice against lethal YFV challenge. In non-human primates (NHP), the S0 vaccine also induced high levels of antibodies, particularly neutralizing antibodies, and decreased viral replication. In a second hamster experiment, a high single dose of vaccine (10^4 PFU) produced strong antibody responses and protection in as little as 10 days. These results were published in *Nature*.

Session 2: pandemic preparedness

2021 Gertrude Elion memorial award lecture: innovation in the quest for treatment, prevention, and cure of viral disease. William Lee, Ph.D., Gilead Sciences, Foster City, California, USA

William Lee reflected on thirty years of antiviral discovery and development at Gilead Sciences. As Head of Research for over twenty years, he defined innovation as the fusion of ideas, invention, and execution that comes together to create a product that improves the human experience. Innovation has many origins and takes focus, time, resources, and passion. Over the past three decades, such innovations have led to treatments for influenza, hepatitis B virus (HBV), HIV, and SARS-CoV-2 infections, as well as to the cure for hepatitis C virus (HCV). To illustrate his personal experience in antiviral innovation, he reviewed the evolution of single-tablet HIV drug discovery and treatment**,** including Atripla in 2006, Stribild in combination in 2012, Genvoya in 2015, and Biktarvy in 2018. William emphasized that besides developing new medicine, innovation should also include access to medicine in low- and middle-income populations. Finally, he presented some exciting results on using a broad neutralizing antibody (PGT121) and TLR7 agonist to induce a simian-human immunodeficiency virus (SHIV) cure in NHPs.

Antivirals and preparedness for pandemics: what we have and what we need. Tomas Cihlar, Ph.D., Gilead Sciences, Foster City, California, USA

Tomas Cihlar discussed how countermeasures for pandemic preparedness should include surveillance,

diagnostics, prevention, and treatment. Besides influenza virus, the WHO has prioritized other viral pathogens with pandemic potential, including filoviruses (Ebola and Marburg viruses), coronaviruses (MERS-CoV, SARS-CoV-1, and SARS-CoV-2), paramyxoviruses (Nipah virus and henipaviruses), arenaviruses (Lassa virus); flaviviruses (Zika, dengue, West Nile, and yellow fever viruses), alphaviruses (chikungunya virus), poxviruses (smallpox virus), and "disease X," the next emerging pathogen. He reviewed (*i*) the available treatments for WHO priority pathogens; (*ii*) two regulatory approval paths for medicine (regular approval based on safety and efficacy from clinical trials and "Animal Rule"); (*iii*) then-current COVID-19 therapy (antibody cocktails and small molecule drugs such as remdesivir); (*iv*) two complementary approaches for developing antivirals (antibody and small molecule inhibitors); (*v*) antiviral approaches targeting viral proteins and host proteins/pathways; (*vi*) options for developing virus-specific and broad-spectrum antivirals; and (*vii*) pros and cons of repurposing clinical drugs for new indications. To achieve end-to-end drug discovery, consortia, coalitions, and partnerships are needed to coordinate funding and execution between government, industry, academia, and non-profit organizations.

Scientific basis for the rapid development of highly effective RNA-based COVID-19 vaccine. Philip Dormitzer, M.D., Ph.D., Pfizer, Pearl River, New York, USA

Philip Dormitzer reviewed the lightspeed journey of Pfizer/BioNTech COVID-19 vaccine (BTN162b2) development. Five innovations have contributed to the rapid development of the COVID-19 mRNA vaccine: synthetic biology, RNA platform, lipid nanoparticles for RNA delivery, spike antigen stabilized in the prefusion conformation, and innovative clinical trials in both USA and Germany using different age groups (18–55 and 65–85 years of age) in parallel. The speed of vaccine development is essential for rapid control of epidemics and pandemics. For example, vaccine development was too slow during the 2009 H1N1 pandemic. Philip showcased Novartis's pre-developed self-amplifying RNA platform, which allows quick insertion of a viral antigen, that was deployed when responding to the H7N9 flu outbreak in 2013, just eight days after the viral sequence became available. Finally, he discussed the approaches to tackle then newly emerged SARS-CoV-2 variants to safeguard the efficacy of COVID-19 vaccine. The neutralizing antibody results, together with real-world effectiveness, suggest that BTN162b2 vaccine remained efficacious against those variants. If needed, boosting with the same BTN162b2 vaccine could enhance immune protection against new variants.

SARS-CoV-2 biology and countermeasure development. Pei-Yong Shi, Ph.D., University of Texas Medical Branch at Galveston, Galveston, Texas, USA

Pei-Yong Shi presented two reverse-genetics systems of SARS-CoV-2, an infectious cDNA clone and a trans-complementation system for single-round SARS-CoV-2 infection. The single-round SARS-CoV-2 infection system has the potential to be used at biosafety level 2 (BSL2), which would accelerate research on SARS-CoV-2 antivirals, vaccines, and virology by opening the field to researchers without access to BSL3 containment. Using these systems, Pei-Yong's team has developed reporter SARS-CoV-2 constructs expressing luciferase and other reporter genes, enabling high-throughput antiviral screening and testing neutralizing antibodies. These assays enabled the rapid development of Pfizer/BioNTech's vaccine. These genetic systems have also allowed studies of the biology of SARS-CoV-2 variants and efficacy of vaccine-elicited antibody neutralization against these variants. For example, the D614G substitution in the spike protein was the first prevalent variant discovered in SARS-CoV-2. Using hamster and human primary airway cultures, Pei-Yong and his collaborators showed that this substitution increases viral replication in the upper respiratory tract in infected hamsters, promoting viral transmission. Their results also showed that the spike N501Y substitution of variant B.1.1.7 (alpha), the prevalent variant at the time, enhanced viral spike/hACE2 receptor affinity, leading to increased viral transmission.

EIDD-2749, a broad active ribonucleoside analog that is highly efficacious in animal model of arenaviral disease. Brian Gowen, Ph.D., Utah State University, Logan, Utah, USA

Lassa fever and other arenaviral haemorrhagic fevers are listed as priority viral diseases by the WHO. **Brian Gowen** showed that EIDD-2749, a uridine ribonucleoside analog, is a broad-spectrum antiviral with good oral pharmacokinetics and once daily dosage. EIDD-2749 has EC_{90} of 3.4 nM and 6.2 nM against Tacaribe virus and Junin virus in Vero cell culture. Doses of 10 mg/kg EIDD-2749 fully protected Tacaribe virus-infected AG129 mice from death even when treatment was initiated 7 days post exposure. Infected animals treated with the compound had undetectable viral loads in organs and sera. Moreover, even treatment with as low as 0.5 mg/kg EIDD-2749 fully protected AG129 mice infected with Tacaribe virus. Additionally, treatment with 10 mg/kg EIDD-2749 starting 7 days post exposure fully protected AG129 mice infected with Junin virus from death, viremia, disease, and weight loss. Overall, the study

shows that EIDD-2749 is a promising potential therapeutic for treating disease caused by arenaviruses.

Replication of alphaviruses: molecular mechanism? Dahai Luo, Ph.D., Nanyang Technological University, Singapore, Singapore

To review alphavirus replication, **Dahai Luo** first presented the structures and functions of viral nsP1 (MTase/GTPase), nsP2 (helicase/protease), nsP3 (with yet undefined roles in replication and interactions with host proteins), and nsP4 (RdRP). Next, he presented the crystal structure of Ross River virus snP4 polymerase domain at a 2.6 Å resolution. The structure showed considerable flexibility, and even conserved domains were disordered. Although the polymerase motifs were not tightly folded, the protein retained robust RNA polymerization activity, albeit weaker than that of dengue NS5. Finally, Dahai presented the cryogenic electron microscopy structure of chikungunya virus nsP1 protein. The structure showed twelve NSP1 molecules forming a crown-like ring with a central channel ~7 nM in diameter that allows the transportation of viral RNA and proteins. The top ring contains the catalytic domains (MTase/GTPase), and the bottom ring contains the membrane-associated domain serving as the neck for assembly of the viral replication complex. These studies are breakthroughs towards obtaining high-resolution structures of the nsP1-4 replication complex, which could provide new approaches for antiviral discovery and rational drug design.

Inhibition of human norovirus replication in cell culture and zebrafish larvae by a novel class of protease inhibitors. Jana Van Dycke, Ph.D., KU Leuven, Leuven, Belgium

No antiviral or vaccine is clinically approved for norovirus treatment and prevention. The goal of the study presented by **Jana Van Dycke** was to identify inhibitors of norovirus. Toward this goal, they found that compound DC40_2267 acts as a viral protease inhibitor. It inhibited the replication of mouse norovirus and human norovirus replicon with EC_{50} of 40 nM and 10 nM, respectively. The compound also showed antiviral activity in a cell-based protease assay. Resistance selection mapped escape mutations to the viral protease. Micro-injection of human norovirus into the yolk of zebrafish larvae showed viral replication, allowing in vivo testing of antivirals. Treating the infected zebrafish larvae with compound DC40_2267 in water led to a reduction of viral RNA. The compound also showed antiviral activity in a mouse model, suggesting that DC40_2267 warrants further development for treating norovirus.

Session 3: COVID-19 vaccines

The coronavirus 2019 (COVID-19) vaccines session was an exciting discussion of both considerations and evaluation of antibody responses to SARS-CoV-2 and vaccines, as well as presentation of the recent data on vaccines developed by Moderna and Janssen.

A perspective on viral vaccine immunity and the issue of disease enhancement. Ann Arvin, M.D., Stanford University, Vir Biotechnology, California, USA

Ann Arvin provided a timely presentation on vaccine enhanced disease (VAED) in light of considerations for COVID-19 vaccine development and use. Broadly, she discussed potential mechanisms of immune enhancement, clinical experience with immune enhancement, and implications for COVID-19 vaccine development. Mechanisms of humoral and cellular immunity that protect against viruses may also theoretically enhance illness when an individual with pre-existing immunity encounters the pathogen; this can occur via antibody-dependent enhancement (Fab-dependent or Fc-mediated) and/or cellular immunopathology (T cell responses that trigger exaggerated inflammatory cytokines or skewed T cell responses that interfere with effective immune control). However, clinical evidence for VAED is very rare. Noted exceptions include antibody-dependent enhancement in rare cases of secondary dengue infection, and VAED in children given formalin-inactivated RSV and measles vaccines in the 1960s. Ann also spoke about influenza, a virus against which pre-existing immunity is only partially protective and natural immunity generally does not result in disease enhancement. She cited the exception of the 2009 H1N1 pandemic, during which cases of potential immune enhancement were reported.

Ann finished by focusing on the implications for COVID-19 vaccine development. She provided evidence that cross-reactivity between coronaviruses confers a protective effect, and as additional support for the benefits of pre-existing immunity, she noted that more rapid adaptive immune responses correlate with improved outcomes. She discussed multi-inflammatory syndrome in children, which was poorly understood early during the early months of the COVID-19 pandemic; however, the data do not suggest that aberrant adaptive immune responses induce this disorder. Ann emphasized that preventing SARS-CoV2 infection remains the best approach. Antibody- or T cell-dependent responses have not been associated with any negative findings in COVID-19 vaccine studies up to this point, and no immune pathology was associated with vaccine candidates under investigation.

Importantly, with the above examples, Ann also discussed the limitations of in vitro and animal model systems in investigating VAED potential. She emphasized that VAED due to pre-existing immunity cannot be

differentiated by clinical signs or biomarkers. Therefore, evaluating the incidence and respective risk of these events requires rigorous, large-scale clinical trials to assess vaccine efficacy and adverse events, and these evaluations must continue in post-licensing surveillance efforts.

Antibody responses to the SARS-Cov-2 spike protein. Florian Krammer, Ph.D., Icahn School of Medicine at Mount Sinai, New York, USA

Florian Krammer lectured about antibody responses to SARS-CoV-2 spike protein after infection and vaccination. He started with a review of the structure of the virus, focusing on the spike protein, and described how his group had started optimizing antibody tests very early. By February 2020 an ELISA test was ready for clinical use. The test proved highly specific and sensitive, detecting 99.5% of the PCR + donors and about 40% of the suspected positives. More than 100,000 donors were screened by October, with some 30,000 scoring as positive, of whom more than 90% had robust anti-spike antibody responses. The ELISA titres correlated well with neutralizing antibody responses and showed that anti-spike antibodies are stable for ~3 months, declining somewhat by 5 months to stable levels until stabilizing at month 7. Not surprisingly, the levels of antibody responses were highly heterogenous among individuals. Florian discussed protection against reinfection in NHPs, with little detectable SARS-CoV-2 RNA in the upper respiratory tract and none in the lungs. However, no antibody titer correlates of protection are yet known for SARS-CoV-2 as there are for other viral infections or toxoids. Florian then described the longitudinal PARIS cohort that was originally designed to follow the long-term immunology of infected patients; when the SARS-CoV-2 vaccine became available, this cohort became well suited for evaluating the effects of vaccines in recovered individuals. In brief, one dose of mRNA vaccine increased and homogenized the antibody titres in all recovered patients, reaching high titres, while the second dose did not provide obvious additional benefit. The ratio of neutralizing to total antibodies was lower in vaccinated persons; however, this resulted from increased levels of both neutralizing antibodies and total antibodies, with the later increasing more, and thus poses no obvious concern. Florian completed his talk by discussing variants of concern and their effects on neutralizing antibodies. No simple relationship is evident between variants and neutralization, with some variants being more or less resistant to neutralization by some antibodies. Interestingly, the N-terminal domain (NTD), not the receptor binding domain (RBD), was identified as the most important for the ability of antibodies to neutralize the virus. Florian's talk presented a tour-de-force effort to develop the tools for evaluating serological responses and protection against infection and to apply these tools to a rapidly progressing pandemic, garnering important and useful data used for decision making in real time.

Janssen's effort in the development of an Ad26-based COVID-19 vaccine, Hanneke Schuitemaker, Ph.D., Janssen Vaccines and Prevention, Leiden, Netherlands

Hanneke Schuitemaker presented the development of Janssen's SARS-CoV-2 vaccine, Ad26.COV2.S. This vaccine is based on the adenovirus Ad26 platform, which uses a non-replicating vector to shuttle genes of interest into the recipient. Various sequence modifications of the SARS-CoV-2 spike protein were evaluated, including those optimizing expression, immunogenicity, and manufacturability. Variations in signal peptides and prolines and the use of membrane-associated or soluble protein were investigated. The final construct was based on the pre-fusion structure of the spike protein and included a mutation of the furin cleavage site, a stabilizing double proline substitution in the hinge region, and a wild-type signal peptide; the final construct is a membrane-associated protein.

Extensive testing in preclinical models, including NHP and Syrian hamsters, demonstrated safety and efficacy as well as assisted in dose determination. In the NHP model, infectious virus was found in lungs of all infected, unvaccinated animals, but in none of the 6 vaccinated animals. In hamsters, significant weight loss (20% or more) was seen in all unvaccinated animals after viral challenge, but no weight loss was observed in animals vaccinated with either dose evaluated. Lung tissue from unvaccinated infected hamsters showed virus and infiltrating immune cells, while neither was seen in vaccinated hamsters. Importantly, no vaccine-associated respiratory disease was observed.

The design of the phase 1/2a study was complex and meant to provide sufficient data at the interim analysis to support the start of a phase 3 study. There were 3 cohorts; cohort 1 included 400 subjects aged 18–55 to demonstrate safety and immunogenicity, cohort 2 included 270 subjects aged 18–55 to study duration of response and boosting, and cohort 3 included 375 subjects 65 and older to demonstrate safety and immunogenicity in this age group. Overall, the profile at interim analysis showed acceptable safety and immunogenicity in all age groups, with lower reactivity in cohort 3. Immunogenicity was demonstrated by ELISA, neutralizing antibody assessment, antibody-dependent cellular phagocytosis, and $CD4^+$ and $CD8^+$ response assessment.

The phase 3 ENSEMBLE study started on September 21, 2020, with administering a single dose of 5×10^{10}

viral particles. The study was conducted on three continents and eight countries, including Brazil and South Africa, where emerging variants of concern beta and gamma were present at the time. The key findings included 66% vaccine efficacy in protecting against moderate to severe disease in all countries starting 2 weeks post vaccination. In the US, vaccine efficacy was 72% against moderate to severe disease, and globally, the study found 85% protection against severe disease. Protection levels were similar in South Africa, which at the time of the trial had high circulating levels of B.1.351 (beta) variant. Vaccine efficacy was similar across ages, comorbidity status, gender, race, and ethnicity. Acceptable vaccine safety was observed, with most adverse events being mild to moderate and resolving in 1–2 days.

Hanneke noted that Janssen has a significant focus on ensuring global access to this vaccine. She noted that it takes many partners to make such a rapid and robust development program successful, citing many of the same collaborators mentioned by Tal Zaks.

Overview of Moderna COVID-19 vaccine: safety, immunogenicity and efficacy, Tal Zaks, M.D., Ph.D., Moderna, Boston, Massachusetts, USA

Tal Zaks gave an update on the preclinical and clinical development of Moderna's mRNA-based COVID-19 vaccine. Prior to the pandemic, Moderna had developed multiple viral vaccine programs, including those against influenza virus, RSV, Zika virus, chikungunya virus, human metapneumovirus/human parainfluenza virus 3, and cytomegalovirus, which provided the company with tremendous experience and allowed them to rapidly apply their platform to developing a COVID-19 vaccine. Tal asserted that this was essentially a "digital medicine" approach, as time from sequencing the virus to initiating vaccine development was 48 h. Working closely with NIH and the FDA in their phase I studies, Moderna scientists were able to rapidly acquire the data needed to support phase 2 studies. Initial safety data from phase 1 informed phase 2 study design, and dose determination from phase 1 triggered implementation of the phase 2 studies. Phase 1 studies demonstrated a tolerable safety profile of the vaccine and showed consistent development of neutralizing antibody titers after a single dose, with titers increasing even further after the second dose. The response was independent of age and, on average, post-vaccination antibody titers were higher than antibody levels in convalescent serum.

Phase 3 studies had a simple design and were executed with strong collaboration with NIH and FDA. Entry criteria included individuals at high risk of infection, encompassing a significant proportion of patients over 65 and those with comorbid conditions, and who were representative of US demographics. Tal noted the difficulty of balancing the desire for a rapid trial with including individuals that fully reflect the diversity of the US population. Analysis of the study data revealed an overall 94.1% efficacy rate, with data at the time of analysis demonstrating 100% protection against severe disease. Of note, data from Pfizer's independently developed mRNA-based vaccine phase 3 trial, released a week later, demonstrated efficacy within 1% of the Moderna results. Local adverse events were mostly associated with pain at the injection site. Systemic adverse events included fatigue, headache, myalgia, and arthralgia; these effects increased with the second dose, but were transient, expected, and correlated with the immune response. Thus, the safety profile was determined to be tolerable enough to warrant use for clinical benefit.

A significant attribute of the Moderna mRNA vaccine is its relative stability. The vaccine is stable for 6 months at −20°C, for 30 days at 4°C, and for 12 h at room temperature. Additionally, a multi-dose vial is stable for 6 h after the first puncture.

At the time of the talk, millions of individuals had been dosed with real-world data confirming the tolerability and efficacy seen in the phase 3 study. Evaluation of efficacy against variants of concern B.1.1.7 (alpha), B.1.351 (beta), and P.1 (gamma) also had shown protection, with neutralizing titers against B.1.1.7 equivalent to titers against the targeted original Wuhan variant. Neutralizing titers against beta and gamma were 6-fold lower than against the Wuhan variant, but were still higher than those in convalescent sera. Moderna was preparing a new vaccine based on the beta variant as a booster for those previously vaccinated, as well as a primary vaccine.

Tal noted that this rapid success in vaccine development could only be achieved with significant collaboration amongst many individuals and organizations, including numerous ISAR members and other investigators and organizations, such as BARDA, OWS, a diversity and inclusion panel, principal investigators, study sites, and study participants.

Overall, like for SARS-CoV-2 antivirals, the rapid, coordinated, and robust efforts to understand the immune response and bring forward safe and effective vaccines has been unparalleled.

Career development interactive workshop: You belong: finding confidence in the face of self-doubt and impostor syndrome. Jen Heemstra, Ph.D., Emory University, Atlanta, Georgia, USA

The annual 2021 ICAR career event was an interactive workshop given by **Jen Heemstra**, a full professor of chemistry with a very popular Twitter account

(@jenheemstra), on which she regularly shares advice on mentoring and science careers.

The topic of the workshop was impostor syndrome, the feeling of doubting one's abilities and accomplishments and feeling like a fraud. Nearly everyone struggles with self-doubt or impostor syndrome in some form at various points in life, but the changes and struggles brought on by the COVID-19 pandemic have exacerbated them. Although eliminating these thoughts entirely may just not be possible, handles and tools to recognize and manage them exist

Jen first reflected on her career path, confessing her own feelings of self-doubt. At times she felt like she was moving backwards, further away from her career goals. However, those times were not wasted: they gave her skills and perspectives that made her even more successful. Her main point was to emphasize that while the career path of others may look like a smooth, straight line, it really is not. She also underscored the importance of having great mentors.

The interactive part of the workshop started after this interesting reflection on Jen's career path. First, participants were asked to think about times when they felt confident in their ability to do something well, how they felt during the activity or event, and whether this confidence and attitude impacted the outcome. Next, participants reflected on times when they doubted their ability do something well, and why they felt that way. Jen pointed out that two things can undermine confidence: what other people tell us and what we tell ourselves. We only have full control over the latter category.

As a tool to refute such thoughts, Jen proposed a framework of facts and stories. Based on facts, multiple stories can be crafted. Jen gave the example of starting a postdoc in a new lab, needing to learn new techniques and not understanding much of what is presented at group meetings. One story that can be made of this situation is, "I am not good enough at research to belong here." However, other facts could tell a different story: "Other group members also had to learn these techniques when they started; I have worked hard before to learn, and I can do it again." With these different facts, one can build a story of belonging and confidence of eventually rising to the level of the other group members.

Jen provided some common stories people tend to tell themselves and solutions for fighting them. For example, we tend to attribute positives to luck and negatives to objective measures. In addition, we tend to overemphasize the importance of the areas in which we are weak while underemphasizing the importance of our strong areas.

Jen concluded that our confidence level can impact our enjoyment of activities and, to some extent, their outcome. Importantly, she emphasized that individuals can change their stories by taking a broader look at the facts. Finally, she advised surrounding ourselves with people who help us to tell positive stories, and to reciprocate such stories for them.

Session 4: COVID-19 therapeutics and diversity speaker award

2021 Diversity speaker award recipient lecture: my career-long fascination with antiviral therapeutics. Craig Cameron, Ph.D., University of North Carolina School of Medicine

Craig Cameron was the recipient of the first ISAR's Diversity Speaker Award, an award developed last year and initially funded by Dr Ann Kwong and Dr Kathie Seley-Radtke. The purpose of the award is to recognize outstanding scientists who have overcome adversity in their careers, including belonging to any demographic underrepresented in science. Craig gave an excellent overview of his career path and his current research focus. He began his career in 1997 at Penn State University and remained there until a few years ago, when he relocated to the University of North Carolina School of Medicine. Throughout his career, he has had two major goals: developing antiviral strategies with broad-spectrum potential and developing strategies based on viral enzyme mechanisms. At the beginning of his career, Craig noted, information about RdRPs, particularly poliovirus RdRP, was limited. To address that gap, he developed a model system that remains an important tool for studying many RNA viruses to this day.

In the 2000s, Craig's attention turned to ribavirin (RBV), an apparently typical nucleoside chain terminator that seemed to have unique properties. His group discovered a new mechanism of action for RBV, termed lethal mutagenesis, adding a second mechanism of action for nucleosidic antivirals. As an extension of those studies, Craig's group also developed a number of new assays to study mitochondrial polymerases, which are typically off targets for nucleoside analogues. As a result, the group was able to pursue new nucleoside analogues that targeted these polymerases directly.

Craig next focused on developing magnetic tweezers, which allowed his group to study nucleotide incorporation by RdRP into extending RNA and its inhibition. Using this tool, he and others were able to obtain incorporation rates over thousands of cycles of addition at single-nucleotide resolution. Using these magnetic tweezers, his group studied nucleosides like favipiravir and remdesivir, observing a phenomenon they termed pausing. Pausing subsequently leads to backtracking from double-stranded RNA to single-stranded RNA, and back to double-stranded, without dissociation. Thus, the group identified a third type of mechanism if RdRP inhibition for nucleoside

derivatives, pausing, in addition to chain termination and lethal mutagenesis.

Finally, Craig discussed his current focus: developing tools for single cell analysis. This approach is not only innovative, but needed due to the genetic heterogenicity of virus and cell populations, variability of drug uptake, metabolism, and other factors. Such tools would allow new insights into infection, replication, antiviral activity, and metabolism of different viruses, as well as infection outcomes for different populations. In short, Craig is an impressive scientist who has long served as a role model for many!

The SARS-CoV1 and 2 replication/transcription machinery and its future for drug-design, Bruno Canard, Ph.D., CNRS and Aix-Marseille University, Marseille, France

Bruno Canard provided an excellent background on viral polymerases and how they have served as one of the best targets for nucleoside antiviral drug design for decades. Unsurprisingly, the RdRP of coronaviruses, including SARS-CoV-2, are currently being studied as antiviral targets. Several nucleosides are being pursued as potential therapeutics against COVID-19. Uniquely among human pathogenic viruses, the coronaviruses a encode proof-reading exonuclease ExoN, nsp14 in SARS-CoV-2, which has rendered many nucleoside analogues ineffective. Despite this challenge, the SARS-CoV RdRP is endowed with several properties that still make it an attractive target. It is at least 10-fold more active than any other known viral RdRP and has unusually high nucleotide incorporation rate. Consequently, it is highly error prone, and some nucleoside analogues, particularly 2′-modified ones, are incorporated efficiently. The RdRP and exonuclease activities lead to a trade-off between insertion ability and the excision rate of the ExoN. Bruno's studies have shown that the active site of SARS-CoV-2 polymerase has some peculiar features that can be exploited in developing antiviral therapeutics.

Among the nucleosides tested against SARS-CoV-2 reviewed by Bruno is favipiravir, a nucleobase that is transformed in vivo to a nucleoside triphosphate. Favipiravir is classified as a lethal mutagenic nucleoside analogue. Another one is remdesivir, which appears to work by several mechanisms including the typical delayed chain termination of the RdRP and by inhibiting second strand synthesis. Interestingly, though remdesivir is an ATP analogue, it is significantly incorporated as a GTP analogue, and is indeed excised by nsp14 ExoN. In addition, some reports also found mutagenic mechanisms for remdesivir. Sofosbuvir was also discussed; however, Bruno mentioned that it is readily discriminated against by the SARS-CoV-2 polymerase and is also excised by nsp14.

Bruno discussed the new ExoN assay developed by his group and its use to search for ExoN inhibitors. The last compound discussed was AT-527 from Atea, which was the focus of **J.P. Sommadossi**'s talk (Session 4). Bruno showed the results of the structural studies currently conducted by his lab, which revealed that two molecules of the AT-527 triphosphate interact with the SARS-CoV-2 polymerase, one at the NIRAN site and the other at the RdRP active site, highlighting how unique this compound is in the fight against SARS-CoV-2.

Development of small molecule protease inhibitors against SARS-Cov-2. Kyeong-Ok Chang, D.V.M., Ph.D., Kansas State University, Kansas, USA

Kyeong-Ok Chang presented a collaborative work between his group and those of Dr. Scott Lovell (Kansas State University), Dr. Groutas (Wichita State University), and Dr. Stanley Perlman (University of Iowa) developing small molecule protease inhibitors against SARS-CoV-2 and other coronaviruses. He started by highlighting the significance of coronaviruses in human health, describing the 3C-Like protease (3CLpro) as an antiviral target, and providing its structural analysis. He highlighted that 3CLpro is a dimer and reviewed its substrate specificity. In over 10 years of previous work with di- and tri-peptidyl compounds, this collaboration had developed the dipeptidyl GC376, which is active against many human and animal coronaviruses and has shown activity in clinical trials against feline infectious peritonitis, a fatal disease produced by a feline coronavirus. An FDA Investigational New Animal Drug had already been filed for using GC376 to treat feline infectious peritonitis at the time of the meeting. Further optimization has been pursued through target, cell-based, and structural assays with a strong focus on the cap modifications. Overall, MERS-CoV and SARS-CoV-2 were more potently inhibited than SARS-CoV by most compounds. Two compounds were tested in infected primary human airway endothelial cells. Several compounds were co-crystalized with MERS-CoV, SARS-CoV, and SARS-CoV-2 3CLpro. The structures show that the cap of one compound interacted differently with the protease of MERS-CoV, which was correlated to higher potency of the compound. One compound was shown to be active in mice infected with a mouse-adapted strain of MERS-CoV. Treatment was effective, though less potent, if started as late as 48 h after infection, but starting later was ineffective. Other compounds were optimized for SARS-CoV-2 and tested in K18 hACE2 transgenic mice. In a model that induces about 50% mortality, treatment resulted in survival of all animals, whereas in a model that induces 100% lethality, 90% of treated mice survived. These studies have shown that dipeptidyl compounds provide a solid foundation for the development of antivirals against coronaviruses, including SARS-CoV-2.

Targeting the proteases of SARS-Cov-2 and other RNA viruses. Christoph Nitsche, Ph.D., Australian National University, Canberra, Australia

Christoph Nitsche discussed targeting the proteases of several RNA viruses, particularly flaviviruses, alphaviruses, and now coronaviruses. He began by describing Zika virus NS2B-NS3 protease in detail, then discussing boronic acid peptide derivatives that potently inhibit NS2B-NS3 of Zika, dengue, and West Nile viruses. However, NS2B-NS3 cleaves a dibasic peptide, which poses a challenge to developing a peptide-based inhibitor with good activity in cells. Using novel Click chemistry, Christoph's group developed a platform to generate and screen macrocyclic peptides as an alternative class of high affinity NS2B-NS3 inhibitors. The best compound had a K_i of 0.14 μM and a half-life of about 20 h in the presence of the protease. Nonetheless, the protease activity has to date precluded co-crystalizing the protease with the macrocyclic uncleaved inhibitor.

Christoph then discussed de novo selection of NS2B-NS3 inhibitors using mRNA display, in which an RNA library is translated while the peptide remains attached to the RNA encoding it via puromycin ligation. The peptides include unnatural amino acids and cyclize spontaneously. Interestingly, none of the enriched peptide sequences that were selected to be synthesized in larger scale and tested bound to the active site. Two types of molecules were identified. Some were potent inhibitors acting most likely via an allosteric inhibition mechanism. Other compounds bound to the active site with high affinity but did not inhibit. Christoph then moved on to discuss the structural similarities between 3CLpro of SARS-CoV, MERS-CoV, and SARS-CoV-2, as well as the commonalities of their cleavage sites, which are mostly uncharged. He also discussed the dimerization interface of 3CLpro as a potential drug target. Next, Christoph reviewed several published SARS-CoV-2 3CLpro inhibitors and their properties, highlighting that all of them attach covalently. Rational design of macrocyclic peptide inhibitors that cannot covalently attach themselves to the protease proved challenging. The group also attempted the above de novo selection approach, which had successfully identified inhibitors of Zika virus protease, and found some inhibitors made of canonical amino acids that need to be further characterized and improved.

AT-527, a double prodrug of a guanosine nucleotide analog, is a potent inhibitor of SARS-Cov-2 in vitro and a promising oral antiviral for treatment of COVID-19. Jean-Pierre Sommadossi, Ph.D., Atea Pharmaceuticals Inc, Boston, Massachusetts, USA

Jean-Pierre Sommadossi presented new biological data for AT-527, a new nucleoside/tide therapeutic entering Phase 3 clinical trials for COVID-19. As his group's studies have shown, AT-527 offers several advantages over remdesivir, another nucleoside analogue in the same mechanistic class which has received emergency authorization for treating COVID-19. Both drugs disrupt viral replication by inhibiting the RdRP. Remdesivir must be administered intravenously, while AT-527 is orally bioavailable. Moreover, levels of the triphosphate of AT-527 (AT-9010) are 7-fold higher in human epithelial cells than those of triphosphate remdesivir. In addition, AT-527 has a favorable safety profile, enhanced potency, and better selectivity. Also of importance, the synthetic route to produce AT-527 is quite facile and highly amenable to scale-up, whereas remdesivir is produced via a tedious synthetic route and scaling up its production has been challenging due to the hazardous steps needed to add the 1′-cyano group.

AT-527 is similar to sofosbuvir, as both compounds feature a fluorine and a methyl group at the 2′-position of the nucleoside scaffold, and both are McGuigan ProTide prodrugs. However, the nucleobase of AT-527 possesses a diamino functionality, with the N6-NH_2 group masked by a methyl group. This group essentially serves as a second prodrug moiety, as it is removed in vivo to yield the guanosine analogue. This is a highly advantageous feature of this nucleoside, as other O-alkylated nucleoside analogues heretofore have exhibited significant mutagenic toxicity, while the AT-527 N-alkyl diamino base has not. Finally, in addition to being active against COVID-19, AT-527 has also shown pan-genotypic activity against HCV and, in combination with daclatasvir, is more effective and can potentially be given for less time than sofosbuvir. The first HCV patient enrolled in the phase 3 clinical trial started treatment with AT-527 on April 30th, 2021, just before this virtual ICAR meeting. Jean-Pierre hoped to report the results of the ongoing clinical trials for AT-527 soon.

Multidisciplinary approaches identify compounds as potential new therapeutics for SARS-Cov-2, Benjamin Bailly, Ph.D., Institute for Glycomics, Griffith University, Queensland, Australia

Benjamin Bailly described a drug repurposing screen that used molecular docking to identify compounds that can inhibit the binding of SARS-CoV-2 S to ACE2, followed by biophysical surface plasmon resonance (SPR) testing of selected hits and testing antiviral activity in cell culture. More than 50,000 compounds were screened in silico, identifying several potential binders to ACE2 or to the RBD of the SARS-CoV-2 S protein. Of these hits, Evans, Blue, EGCG, Levodopa, velapatasir and albrutinib scored as ACE2 binders in SPR, and several of them inhibited interactions between S RBD and ACE2. Only a handful of compounds bound to S on virus-like particles as evaluated by SPR, but all of them inhibited the interaction between

SARS-CoV-2 S RBD and ACE2. The group then further evaluated the efficacy of selected compounds to inhibit infection of Vero-E6 cells by an early Australian SARS-CoV-2 isolate closely related to the original Wuhan isolate. Some compounds, including Evans, Blue, and suramin (which had been previously identified as active against SARS-CoV-2) inhibited SARS-CoV-2 infection with EC_{50} in the tens of micrograms, and two others, lifitegrast and lumacaftor, also inhibited infection but only at higher concentrations. Other compounds that inhibit S binding to ACE2 did not inhibit infection. Benjamin finished the talk by discussing current and future work continuing this project.

Session 5: retroviruses and herpesviruses

This session consisted of six outstanding talks that focused, as the name of the session implies, on diseases produced by retroviruses and herpesviruses, and on treatments of such diseases. The talks can be divided into two categories based on the virus discussed. We will first summarize talks covering herpesviruses, followed by the lectures discussing retroviruses.

HSV-1 and Alzheimer's disease: causation or association? Understanding the biologic plausibility. Christine Johnston, M.D., University of Washington, Seattle, Washington, USA

Christine Johnston who provided sound evidence for the role that herpes simplex virus 1 (HSV-1) may play in the development of Alzheimer's disease (AD). The hypothesis that HSV-1 may contribute to or even cause AD has been proposed for decades, but actual causality has yet to be established. While Christine's talk did not discuss evidence that establishes actual causality, she did provide evidence from multiple disciplines (epidemiology, in vitro studies, and animal models, to name the most important ones) that strengthen the case for a causal link between HSV-1 and AD. One of the more prominent examples demonstrates that HSV-1 infection is associated with amyloid beta production and induces abnormal phosphorylation of tau protein, both of which are associated with the development of AD.

New helicase-primase drug candidates with sufficient target tissue exposure affect latent neural herpes simplex virus infections. Gerald Kleymann, Ph.D., Innovative Molecules GmbH, Bad Salzuflen, NRW, Germany

Gerald Kleymann provided information about IM-250, a helicase-primase drug for the treatment of HSV infections. IM-250 is ~100-fold more potent in vitro against wild-type HSV than the current standard of treatment (acyclovir). In addition, this drug maintains a high level of activity against HSV-1 isolates in a lethal challenge mouse model and against HSV-2 isolates in a non-lethal guinea pig model. IM-250 has a long in vivo half-life, as well as good bioavailability, excellent target tissue exposure, and a broad volume of distribution—all excellent qualities in a drug for clinical use.

The molecularly engineered H84T banLec has broad-spectrum antiviral activity against human herpesviruses. Megan Lloyd, Ph.D., SUNY Upstate Medical University, Syracuse, New York, USA

Megan Lloyd discussed the potential for H84T BanLec, a molecularly engineered lectin, as treatment for diseases caused by the herpes family of viruses. H84T BanLec binds to N-linked glycans on the viral envelope, preventing viral entry, uncoating, and spread. Since N-linked glycans are present on the envelope of herpesviruses, this compound was postulated to inhibit these viruses. Indeed, H84T BanLec demonstrated broad-spectrum anti-herpesvirus effects with little to no observed toxicity in vitro. In addition, H84T BanLec was as effective as cidofovir (CDV) against varicella zoster virus (VZV) in vivo but was better tolerated. Further experimentation demonstrated that H84T BanLec prevented herpesvirus spread but not virion production in infected cells.

Oral USC-373, an HPMPC prodrug, prevents varicella zoster virus replication in a mouse model. Jennifer Moffat, Ph.D., SUNY Upstate Medical University, Syracuse, New York, USA

Jennifer Moffat discussed data demonstrating that oral USC-373, a CDV analog/prodrug, is highly potent against VZV (both wild-type and acyclovir-resistant variants). In addition, USC-373 prevented replication and spread in vivo compared to vehicle controls regardless of route of administration (injection or oral). Importantly, oral administration of the drug had highly significant, long-lasting effects on viral replication even at the lowest doses.

Barriers to curing HIV infection. Robert Siliciano, M.D., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Robert Siliciano discussed the problems associated with curing HIV infections. Current anti-retroviral therapy prevents HIV replication but does not eliminate the latent reservoirs in the long-lived memory CD4 T-cells from which the virus can rebound, resulting in disease progression. Thus, any curative HIV measure must include a means to remove or even eliminate extremely stable latent HIV reservoirs. To achieve this goal, certain fundamental

parameters must be evaluated, such as accurately measuring reservoirs with intact proviruses that can cause infection separately from defective proviruses that cannot. In addition, the majority of cells in the reservoir are not generated as a result of new infections, but rather by proliferation of previously uninfected cells. Unfortunately, proliferation of these cells is controlled by normal antigenic responses rather than viral control, meaning that suppressing their proliferation can result in a severely compromised immune system.

Lenacapavir: a first-in-class phase 2/3 HIV capsid inhibitor with potential for twice yearly dosing. Jennifer Zhang, M.Sc., Gilead Sciences, Inc, Foster City, California, USA

Jennifer Zhang discussed the use of lenacapavir as a long-term (low-dosing) agent. After several rounds of chemical modifications to optimize potency and metabolic stability, the result was a compound that affects capsid function at multiple points during the HIV lifecycle and can potentially be used as a long-lasting therapeutic option. The ultimate driver for this project is improving patient compliance, through the requirement of only an injection once every 6 months instead of taking a pill daily without interruptions.

Session 6: other viruses and women in science speaker award

Women in science speaker award recipient lecture: genetic diversity and evolution of herpesviruses. Graciela Andrei, Ph.D., Rega Institute for Medical Research, Leuven, Belgium

Graciela Andrei provided an overview of herpesviruses, classical anti-herpesvirus agents, and new antivirals with novel targets (helicase-primase inhibitors, terminase inhibitors, and a UL97 protein kinase inhibitor). She discussed that failure of antiviral therapy is largely due to presence of mutations in viral kinases and herpesvirus DNA polymerases, and that most resistant mutations map to conserved regions of these viral genes. She then provided a summary of factors leading to herpesvirus drug resistance, including viral factors, immune suppression, host factors, and drug factors.

Graciela suggested viewing antiviral drug resistance as an evolutionary process and emphasized what can be learned from translational drug resistance research. She then presented work with the Research Group for Antiviral Resistance (RegaVir) platform, which provides rapid genotyping and/or phenotyping of clinical isolates to analyse different aspects of drug resistance (e.g., novel mutations, multi-drug resistance, compartmentalization, dynamic evolution, heterogeneity). The aim is to identify viral drug resistance as reason for therapy failure, optimize antiviral therapy, avoid drug toxicity, improve patient care, and reduce cost of antiviral treatment. Graciela used several patient-based examples to demonstrate the utility of the platform in providing insights into herpesvirus diversity and rapid evolution in the immunocompromised host, and into adjusting therapy. She highlighted the advantage of next-generation sequencing (NGS) to detect minor viral populations and emergence of drug resistance. She provided other examples in which RegaVir identified contributors to increased risk for developing (multi)drug resistance in immune-privileged sites, compartmentalization of (multi)drug resistance, and simultaneous and concomitant herpesvirus infections.

Graciela completed the talk by discussing her laboratory work investigating a novel mechanism that may contribute to herpesvirus genetic diversity: mutations in DNA polymerase (DNApol) that affect DNA replication fidelity. Her lab identified two novel amino acid changes in the C297W (3′–5′ exonuclease domain) and C981Y (thumb domain) of murine gammaherpesvirus 68 (MHV-68) DNApol related to a mutator phenotype; association of C297W with a mutator phenotype was validated by CRISPR/Cas9 genome editing. Finally, studying population evolution by NGS indicated that the competitive fitness of MHV-68 mutator phenotype viruses with and without antivirals was significantly impaired.

Antiviral agents for serious RNA virus infections; a personalized medicine approach. Judith Breuer, M.D., Institute of Child Health UCL, London, UK

Judith Breuer discussed the challenges and approaches to clinical treatment of *Othornaviridae* infection, including measuring efficacy in vivo and finding the cause of failed treatment in a talk entitled, *Antiviral agents for serious RNA virus infections; a personalized medicine approach*. Favipiravir is an RdRP-targeting drug with known liver toxicity but broad-spectrum antiviral activity. It acts as a chain terminator when administered at high concentrations, and has a lethal transition mutagenesis effect when administered at low concentrations. Judith's group aimed to evaluate if favipiravir treatment was associated with clinical improvement and if a biomarker could be used to identify any clinical improvement. Treating patients infected with norovirus, influenza B, or RSV indicated that clinical improvement coincided with detection of lethal mutagenesis in these viruses. To better understand how and why mutational frequency was associated with clinical improvement, a mathematical model of the RSV mutational threshold was developed, showing that ~11 mutations/genome resulted in a 4–8 fold reduction in viral fitness. Finally, in collaboration with Dr Joana Rocha-Pereira's laboratory, a

hamster model of SARS-CoV-2 infection was developed to address whether loss of fitness correlated with clinical improvement. The collaborators observed that low-frequency C→T and G→A mutations in the virus increased over time in a dose-dependent manner when the hamsters were treated with favipiravir. No change in viral load was detected, but a dose-dependent loss of viral replication (fitness) and decreased host tissue damage were observed. Overall, Judith concluded that favipiravir has a modest but important effect on clinical status; treatment with favipiravir is associated with increased mutagenesis; mutagenesis is shown in models to reduce viral fitness; and even significant reduction in viral fitness is not associated with large reductions in viral load.

Judith continued by discussing combination therapy using an example of influenza B treatment with favipiravir and zanamivir, in which favipiravir was found to work synergistically. Finally, she discussed studies of remdesivir treatment in patients, which did not produce evidence of mutagenesis; remdesivir was found to suppresses viral load in some patients but not in others. She emphasized the benefit of remdesivir but also the need for combination therapy to overcome tissue penetration limitations of remdesivir used alone. Overall, RdRP inhibitors have the potential to act as broad-spectrum antiviral agents against RNA viruses, though their clinical benefit may not be associated with reduced viral loads. Instead, some RdRP inhibitors induce lethal viral mutagenesis; lethal mutagenesis levels of ~15–20% appear to be associated with clinical improvement and reduction in viral fitness. Finally, combination therapy, including combinations with other RdRP inhibitors, is likely to produce synergistic effects.

Four-segmented Rift Valley fever virus as a novel live-attenuated vaccine for animal and human use. Jeroen Kortekaas, Ph.D., Wageningen Bioveterinary Research; BunyaVax, Lelystad, Netherlands

Jeroen Kortekaas presented ongoing work on a novel Rift Valley fever virus (RVFV) vaccine platform in a talk entitled, *Four-segmented Rift Valley fever virus as a novel live-attenuated vaccine for animal and human use*. He first reported on innovations in animal model development, describing a new model system in which RVFV is transmitted from lamb to lamb by laboratory-reared *Aedes aegypti* mosquitoes. These models can be used to study mosquito-mediated transmission and to evaluate vaccine efficacy. He then introduced the four-segmented RVFV vaccine platform being developed for human (hRVFV-4s) and veterinary (vRVFV-4s) use. The vaccines are generated by splitting the M genome segment into two M-type segments, each encoding one of the two structural glycoproteins, Gn or Gc. For added safety, the NSs gene is either completely deleted (vRVFV-4s) or partially deleted (69%, hRVFV-4s). RVFV-4s replicates efficiently in cell culture but is completely avirulent in mice (intraperitoneal or intranasal inoculation models), pregnant ewes, and lambs (intramuscular and subcutaneous inoculation). Jeroen presented a series of studies demonstrating that vRVFV-4s does not disseminate in vaccinated animals, sheds or spreads to the environment, or reverts to virulence. Single-dose vaccination efficacy was evaluated in lambs, goats, calves, and pregnant ewes; such vaccination induced protection against homologous and heterologous challenge.

Based on the success of the veterinary vaccine, the Live-Attenuated Rift Valley Fever Vaccine for Single Shot Application (LARISSA) consortium was formed to evaluate the platform for human use. The human candidate vaccine was shown to be immunogenic and efficacious in both mouse and lamb models. Safety studies in marmosets showed no weight loss in vaccinated animals. However, vaccinated animals developed elevated body temperatures that were dose-dependent and began 24 h earlier than in control animals infected with wild-type RVFV. This observation was ascribed to the robust innate immune responses to vaccination and was supported by concurrent neutrophilia in vaccinated animals. In conclusion, hRVFV-4s was found to be safe in marmosets, was not shed or spread to the environment, and induced a rapid innate immune response and neutralizing antibodies within 7–14 days after single-dose vaccination. Altogether, RVFV-4s-derived vaccines were shown to be safe and efficacious, supporting their continued development for both animal and human use.

Understanding the multiple functions of the bunyavirus polymerase protein. Maria Rosenthal, Ph.D., Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Maria Rosenthal summarized current knowledge of the role of bunyavirus L protein, a multi-functional and multi-domain protein that contains the viral RdRP and serves as a good drug target, in the talk entitled, *Understanding the multiple functions of the bunyavirus polymerase protein*. She provided helpful analyses comparing bunyavirus L protein with the polymerase complexes of other viruses (e.g., influenza virus, order *Articulavirales*). Maria emphasized that while our knowledge of L protein structure and functions has increased over the years, it remains a challenging target due to its size and flexibility, as well as its diversity among bunyaviruses.

Maria described the advances in our understanding of the L protein structure and functions attained by her lab through studies combining biochemistry, structural biology, and virology methods. By solving structures of a C-terminal domain in the L protein using X-ray crystallography, the group identified a cap-binding domain essential for viral transcription. She highlighted that the details of

cap binding differ within the order *Bunyavirales* and compared to other viruses, even those with structurally similar polymerases. Furthermore, her group established expression and purification procedures for full-length L proteins to investigate their structure using single-particle cryo-electron microscopy and characterize their diverse functions in biochemical assays. These results, along with data published by other groups, demonstrate the functional and structural similarities and differences of the L proteins within *Bunyavirales* and compare them to those of influenza virus polymerase complex. These studies furthermore support targeted drug development strategies against bunyaviruses.

Rational modifications and biological evaluation of novel non-nucleoside inhibitors of the viral polymerase with antiviral activity against norovirus. Salvatore Ferla, Ph.D., Swansea University, Swansea, Wales, UK

Salvatore Ferla discussed work on the development of novel norovirus antivirals that target the RdRP in a talk titled, Structure and ligand-based virtual screening using different RdRP crystal structures of human and murine norovirus identified two compounds that inhibit RdRP in the micromolar range, though neither showed interesting antiviral activity in cell-based assays due to poor solubility. To improve solubility, rational modifications were introduced in the hydrophobic ring portion of these molecules, including inserting carboxylic substituents in ring 1 and replacing with heteroaromatics in ring 2. These changes improved water solubility without affecting the polymerase-inhibitory activity of the compounds, yet no antiviral activity was observed in cell-based assays. To confer antiviral activity, a scaffold replacement procedure was applied; unfortunately, the new compound only inhibited RdRP by 20% at 100 μM.

A computer-aided flexible alignment approach was subsequently used, identifying the TPB compound as overlapping best with the group's original first-hit structure. Twelve new compounds were designed and synthesized based on combining TBP attributes with key structural aspects from the first hit; four displayed antiviral properties. Two molecules were subsequently selected and analysed by a synthetic chemistry approach. Twenty-three additional compounds were synthesized based on this new scaffolding. The new compounds were very promising antivirals but also unstable in aqueous solution. Since these compounds represent one of the few examples of non-nucleoside polymerase inhibitors with significant antiviral activity against human norovirus replication in a cell-based system, the future goal is to increase their stability and solubility in water. Salvatore's group is also planning mutational studies and the use of *in silico* techniques to design more potent compounds.

Metabolic stabilization of a novel inhibitor of human dihydroorotate dehydrogenase (hDHODH) with potent broad spectrum antiviral activity. Nora Fohrmann, M.S., University of Hamburg, Hamburg, Germany

Nora Fohrmann presented work on improving the metabolic stability of the lead structure in a novel series of compounds with strong antiviral activity against a panel of RNA viruses, including several bunyaviruses, Lassa virus, and Ebola virus, in a talk entitled, *Metabolic stabilization of a novel inhibitor of human dihydroorotate dehydrogenase (hDHODH) with potent broad spectrum antiviral activity*. The antiviral activity of these compounds is based on inhibiting hDHODH, a cellular enzyme involved in de novo biosynthesis of the pyrimidine nucleotides crucial for viral RNA replication. The lead structure of these compounds, containing an elongated alkyl chain, was identified using a hit-to-lead approach evaluating the structure/activity relationship. However, a pharmacokinetic investigation of this lead structure showed metabolic stability (S9, rat) of only 22%, which is insufficient for in vivo evaluation.

To improve stability, the lead compound main metabolite was identified via liquid chromatography–mass spectrometry and the site of metabolism was stabilized via rational derivatization. Three lead optimization cycles were conducted during which the redesigned structures were synthesized and their metabolic stability and enzymatic inhibition were biologically evaluated. The initial cycle of modifications increased stability, but reduced activity mildly to significantly. Of the modified structures, the urea compound was selected for subsequent optimization cycles. The urea analogue was synthesized in 6 steps with good yield; this approach was used to generate further derivatives for the next two optimization cycles. The structure was modified to stabilize against hydroxylation, increasing stability to 90% but resulting in strong reduction of activity. The final optimization cycle focused on reducing IC_{50} by modifying the linker fragment. This improved IC_{50} and only reduced the stability to 70%. Overall, while stability of the lead compound was improved, a decrease in IC_{50} against certain agents (e.g., Lassa virus) was observed in culture. Future work will continue to optimize stability and potency.

Small molecules from the diketo acid class engage and inhibit the endonuclease domain of a panel of bunyaviruses and interfere with viral replication in vitro. Sebastiaan ter Horst, B.S., KU Leuven, Rega Institute for Medical Research, Belgium

Sebastiaan ter Horst discussed the work on the endonuclease domain of RdRP as an antiviral target for bunyaviruses. Structural studies of the domain support the concept that the chelating agent class of diketo acids could serve as starting point for creating broad-acting antiviral candidates. The group found the endonuclease domain to be well conserved,

more so than the polymerase domain, especially within bunyavirus families. This domain is present in and shares sequence similarities with other negative-strand RNA viruses like influenza. Based on this rationale, the group investigated drugs with known activity against influenza as candidates for bunyavirus treatment. Interaction studies were performed using a panel of bunyavirus cap-snatching endonucleases in a thermal shift fluorescence resonance energy transfer- (FRET) based nuclease monitoring assay and GlideSP docking simulations. In addition, in vitro inhibition assays were performed with BUNV-mCherry reporter virus.

The first-in-class FDA-approved diketo acid-based influenza drug baloxavir efficiently bound to the endonuclease of La Crosse virus (LACV), though with lower affinity than it bound to influenza virus endonuclease. Furthermore, baloxavir inhibited BUNV-mCherry replication in vitro with an EC_{50} of 0.7 μM. This inhibition profile was similar to that of RBV, a last-resort option for treating bunyavirus infections, but was at lower compound concentrations. The group also demonstrated a synergistic effect of baloxavir and RBV at certain concentrations. To see if other anti-influenza antivirals may also be suitable for treating bunyavirus infections, the group evaluated L-742,001. Interestingly, a common hydrophobic sub-pocket was identified in binding assays with LACV, Andes virus, and RVFV; L-742,001 and its derivatives demonstrated EC_{50} values between 5.6 and 6.9 μM in vitro.

Overall, this group found that the cap-snatching endonuclease domain is a valid target that can be exploited by chelating the metal ions in the active site of the domain. Future work will focus on rational design of a molecule to better fit the extensive and shallow binding site of bunyaviral endonuclease with the aim of developing efficacious broad-spectrum inhibitors.

Session 7: arboviruses

While the COVID-19 pandemic continues to impact all our lives, the session on arboviruses at the 34th ICAR covered the field of vector-borne viral infections well. The session had three invited speakers -- Dr Jenny Low (Singapore), Dr Johan Neyts (Belgium), and Dr Mark Heise (USA) -- and three speakers selected from the submitted abstracts: Dr Justin Julander (USA), Dr Gerry Rassias (Greece), and Dr Jinhong Chang (USA).

New strategies for modelling and treating emerging viral pathogens. Mark Heise, Ph.D., University of North Carolina, Chapel Hill, NC, USA

Mark Heise provided an impressive update on the use of mouse strains developed in the Collaborative Cross to identify factors that contribute to the severity of chikungunya virus infection. Through these efforts, different host pathways were identified that contribute to different disease phenotypes. These results highlight the contributions of the Collaborative Cross project to further our understanding of the pathogenesis of emerging viruses.

Biologics as therapeutics for rapid response- can we get there fast enough? Jenny Low, M.D., M.P.H., Singapore General Hospital, Singapore, Singapore

Jenny Low shared a perspective of the ongoing arbovirus infections. Singapore experienced its highest ever dengue case numbers in 2020, recording more fatalities than from COVID-19 at that stage. Jenny also discussed the swift bench-to-bedside development of therapeutic antibodies against Zika virus, which was made possible by advances in identifying potent antibodies and development for scale-up of biotherapeutics to be used in human clinical trials together with the adaptive trial designs that allow overlapping studies addressing key clinical development steps. The complexities associated with the roll out of Dengvaxia were touched upon by Drs. Neyts, Low, and Rassias, and the need for effective antiviral small molecules and therapeutic antibodies was discussed.

Pan-serotype dengue virus inhibitor that blocks the NS3-NS4B interaction and exhibits unprecedented in vivo potency. Johan Neyts, Ph.D., KU Leuven, Rega Institute, Leuven, Belgium

Johan Neyts presented exciting data on JNJ-A07, a nM to pM range, pan-serotype inhibitor of dengue virus replication developed together with Janssen Pharmaceuticals. This compound is active in vitro in several cell types. Its mechanism of action appears to involve blocking of NS2B-NS3 interactions with NS4B. The compound was also active in AG129 mouse infection models, including during infections under antibody-dependent enhancement conditions. A high barrier to the selection for resistance was demonstrated. Although escape mutants with signature mutations in NS4B eventually appeared, they arose after months of passaging the virus supernatant. The excellent preclinical data, including compelling pharmacokinetics/pharmacodynamics (PK/PD) information, support this compound, a first-in-class directly acting antiviral against the four serotypes of dengue virus, reaching early phase human trials.

The integrity of yellow fever virus replication Complex maintained by Nonstructural 4B protein and targeted by a small molecule antiviral agent. Jinhong Chang, M.D., PhD., Baruch S. Blumberg Institute

Continuing with the development of inhibitors of nonstructural proteins, **Jinhong Chang**'s talk focused on mechanistic studies of BDAA, a small molecule inhibitor

of NS4B, in yellow fever virus infection. After screening nearly 200 analogs of BDAA, the group discovered a compound with potency in the nanomolar range. Detergent treatment followed by IFA revealed that the mechanism of the compound may involve disruption of the membrane invagination enclosing the viral replication complex to expose the dsRNA, which are then cleaved by cellular RNases. The role in dsRNA protection by NS4B suggests that this protein has a key role in the replication vesicles formed by the replication complex.

Development of a novel dengue protease inhibitor. Gerry Rassias, Ph.D., University of Patras, Department of Chemistry, Patra, Greece

Gerry Rassias described the activity of a NS2B-NS3 prodrug, SP-471P, with low micromolar range activity against all four serotypes of dengue virus. SP-471P inhibits viral RNA replication and production of infectious viral particles even when administered 6 h post infection. Mechanistically, SP-471 appears to inhibit both normal intermolecular protease processes and intramolecular cleavage events at the NS2B-NS3 junction, as well as at NS3 internal sites, which are critical for virus replication. The importance of the internal cleavage site and its dominant negative effect on virus RNA replication appears intriguing and is being investigated in more detail. The protease is a challenging target, but the team is focused on exploiting this new finding and the unique prodrug approach to discover more potent inhibitors.

The ribonucleoside analog EIDD-2749 is broadly active in the treatment of eastern equine encephalitis and chikungunya viral infections. Justin Julander, Ph.D., Institute for Antiviral Research, Utah State University, Logan, Utah, USA

Justin Julander presented work on a potent, broadly active nucleoside analog. Treatment of Eastern equine encephalitis (EEEV) or chikungunya virus infection in mouse models with the ribonucleoside EIDD-2749 resulted in improved outcomes. This compound was also effective when treatment was delayed until 48 h after virus challenge.

The potent small molecule inhibitors targeting nonstructural proteins of dengue virus and yellow fever virus, a nucleoside analog with activity against EEEV and chikungunya virus, and a monoclonal therapeutic antibody against yellow fever virus described by Dr. Low together demonstrate the efforts of the antiviral research community in responding to visible gaps in the availability or access to countermeasures for various emerging viruses. Even when an effective vaccine is available, the need for such countermeasures is still of high importance, as evidenced during the 2016–17 yellow fever outbreak in Angola and Democratic Republic of Congo.

Session 8: hepatitis viruses and William Prusoff memorial award

2021 William Prusoff memorial award recipient lecture: mid-term report on my academic journey to discover novel HBV/HDV therapeutics, David Durantel, Ph.D., INSERM, Lyon, France

David Durantel began his award lecture by profusely thanking his mentors, particularly Dr Fabien Zoulim, and the people who had worked with him, especially Dr Julie Lucifora, highlighting how important these interactions and collaborations have been in his career. He then introduced the major problems resulting from HBV and hepatitis delta virus (HDV) infections, including the approximately 1 million deaths per year, mostly due to the resulting hepatocellular carcinoma. Both viruses persist as nuclear episomes, but HDV is a satellite virus that uses HBsAg-coated particles vesicles to spread between cells. Although there are approved antivirals against HBV and many more are being studied, none can completely eliminate the infection and thus none can fully protect against the residual risk. Several innovative approaches are being pursued to decrease the residual risk, from host-targeting agents with combined antiviral and anticancer activities through nucleic acid polymers or siRNA to decrease antigenemia, to restoring T cell functionality with checkpoint inhibitors or therapeutic vaccines, to epigenetic regulation to lose the cccDNA episomes.

David also reviewed the variety of models used in the development and evaluation of anti-HBV or D antivirals, from cultures of primary human hepatocytes to progenitor cell lines that differentiate into hepatocytes in culture (HepaRG) to mouse models. Chimeric immunodeficient mice expressing hepatotoxic proteins reconstituted with human primary hepatocytes are most useful in evaluating antivirals but also limited because they cannot be used to study immunological processes. Immunocompetent mice can be transduced with adeno-associated virus (AAV) vectors that efficiently deliver the vectored HBV genomes into the liver, thus allowing the evaluation of the roles of immune responses during HBV therapy.

David then discussed the standard antiviral treatments based on the direct acting antivirals (DAA) entecavir, TDF, and TAF. These potent DAA potently reduce viremia while having little effect on HBsAg and cccDNA, although the models suggest they should decrease cccDNA. Considering that HIV/HBV co-infections are common, having nucleosidic prodrugs that only get activated in the liver to treat HBV without risking selection for HIV resistance would be an asset; these nucleosides are a goal of HBV medicinal chemistry. Another goal is

the development of combination therapy using nucleosidic and capsid assembly modulators (CAMs) ; there is thus a strong focus on these inhibitors. CAMs can result in the formation of empty or aberrant capsids, and also have secondary mechanisms of action affecting uncoating and cccDNA establishment, for example. At higher concentrations, capsid inhibitors may inhibit HBeAg secretion. The core antigen HBc also has regulatory functions, having a complex interactome. It plays a role in cccDNA and HBV RNA biology through interactions with both molecules and many host factors including RNA binding proteins (RBPs).

David then discussed the latest developments and directions in HBV antiviral therapy. Capsid inhibitors resulting in aberrant capsids have been found to decrease RNA biogenesis, leading to decreases in pregenomic (pg) and total RNA accumulation, but not in cccDNA, after 75 days of treatment in culture. Consistently, David advocated for combination treatment with long-term nucleosidic and capsid inhibitors to enable mutual potentiation between these two drugs. Another approach would be to inhibit protein kinases that modulate HBc activity and oligomerization. For example, targeting of PLK1, which is involved in HBc phosphorylation and HBc assembly, with small molecules or siRNA inhibits HBV replication in culture or mouse models, and could therefore synergistically complement nucleosidic and capsid inhibitors. Another protein kinase has been recently identified to phosphorylate the capsid and to be required for HBV replication, although the identity of the kinase could not be disclosed at the time. Yet another approach is to use immunomodulators to activate innate immunity, thus replacing interferon. RIG-I agonists were preferred early on, but the adverse effects that resulted in the halting of the clinical trials of inarigivir have since tempered the enthusiasm for this approach. The focus has thus shifted to the Toll-like receptors, mainly TLR2 and 3 (TLR 7 and 8 are not expressed in the liver). Their agonists have been shown to decrease cccDNA to some extent, but their major effect is on the production and accumulation of HBV RNA. An additional advantage of these agonists is that they are also active against HDV. Although their mode of action is still being evaluated, these agonists have shown good activity in the AAV mouse model as nanoparticles with polylactic acid, which home to the liver where they are maintained for weeks. These formulations have proven active in the AAV mouse model and, in contrast to lamiduvine, they resulted in no rebound.

David concluded that combination therapies are needed for HBV and directed our attention to the presentations by John Tavis and Adam Ghering (both Session 8). He indicated the need to target RNA biogenesis, accumulation, and stability, and stated that several drugs achieve these goals, including DAA, immunomodulators, and antimetabolites like the FXR agonist vonafexor, which also inhibit HDV replication (also discussed by Julie Lucifora, see Session 8).

Towards combination treatments for chronic hepatitis B: a virologist point of view. John Tavis, Ph.D., Saint Louis University, Saint Louis, Missouri, USA

John Tavis discussed HBV therapy. HBV is an enveloped, partially double-stranded DNA virus that replicates in hepatocytes. HBV replicates within the viral capsid by reverse transcription of a pgRNA, which is catalyzed by a polyfunctional polymerase that bears both reverse transcriptase and RNase H activities. HBV chronically infects ~250 million people worldwide and results in >850,000 deaths per year. Currently approved HBV therapies include PEGylated IFNα (pegIFNα), which is associated with serious side effects. Nucleos(t)ide analogs (NAs) like lamivudine, adefovir, entecavir, telbivudine, and tenofovir strongly suppress viral replication and normalize ALT levels in most patients. However, they do not fully abrogate disease progression and are life-long treatments, with a functional cure rate below 10% and quasi-universal rebound upon treatment arrest More efficacious curative therapies for chronic hepatitis B (CHB) infections will almost certainly require combinations of multiple drugs acting by complementary mechanisms. The biggest obstacle to curing HBV is the elimination of its nuclear episome, cccDNA, which is the template for all replication intermediates of HBV. It persists in liver cells due to long apparent half-life and replenishment by de novo infection and intracellular amplification. Eliminating or permanently silencing cccDNA is key to curing HBV.

Obtaining a sterilizing cure involving complete elimination of cccDNA seems unlikely at the moment. Therefore, a functional cure that suppresses both HBV DNA and serum-secreted HBsAg by restoring the anti-HBV immune response and reversing disease progression is a more realistic goal for future therapies. Given the excellent safety profile of already approved nucleoside therapies, future curative drugs should maintain this favorable safety profile, both as single agents and combination therapies. To achieve this goal, a very wide variety of treatment strategies are under development. The major classes of drugs being explored include DAA that interrupt production or intracellular maintenance of HBV. The most advanced agents of this class are entry inhibitors, CAMs, siRNAs, and replication inhibitors (mainly improved nucleosides). Host targeting approaches aim to suppress HBV by interrupting cellular mechanisms and immune enhancement by exploiting the power of the adaptive immune response against HBV. At this point, it is unclear which drug combinations are most promising for achieving a functional cure in the highly diverse hepatitis B patient population. Therefore, combinations studies should not

only be rationalized based on preclinical demonstration of synergy, but also be empirically tested in the clinic.

Towards combination treatments for chronic hepatitis B: an immunologist's point of view, Adam Gehring, Ph.D., Toronto General Hospital Research Institute, Toronto, Ontario, Canada

Adam Gehring presented the counterpoint to the virology perspective of the previous lecture in his talk entitled, *Towards combination treatments for chronic hepatitis B: an immunologist's point of view*. One of the major limitations of all DAA is that they only target steps of the viral replication cycle downstream of cccDNA, including viral RNA biogenesis, protein synthesis, and rcDNA production. Therefore, HBV DNA and HBsAg rebound after treatment termination, posing a major challenge even for new DAA candidates like antisense oligonucleotides (ASO) and siRNAs. A coordinated immune response is required for clearance of HBV infection. However, HBV-specific T and B cell immunity display a profile of profound exhaustion in CHB patients. Immunotherapeutic drugs being developed for CHB target both innate and adaptive immunity. These include therapeutic vaccines, checkpoint inhibitors, and small molecules targeting host pattern recognition receptors.

Therapeutic vaccines have the longest history as immunotherapeutic interventions in CHB but have thus far proven ineffective, having no significant impact on HBV replication despite inducing T cell immunity associated with production of anti-HBs antibodies. Newer vaccine candidates currently in early phases of clinical development include DNA vaccines, adenovirus or modified vaccinia vectors, and peptides. These candidates aim at inducing a stronger T cell response with more immunogenic adjuvants. Checkpoint inhibitors are gaining attention, with increasing data on safety profiles from cancer patients, but have only entered small pilot studies. Innate immunomodulators targeting pattern recognition receptors have demonstrated target engagement but only modestly impact viral replication in monotherapy. Selgantolimod (SGLN), a TLR-8 agonist that effectively engages its receptor, induced a strong innate immune response causing a moderate decline in HBsAg levels in a phase-2 clinical study. However, SGLN failed to demonstrate additional reduction of viral DNA over standard-of-care nucleosdic drugs. Similarly, 24 weeks of treatment with TLR-7 agonist GS-9620 induced cytokine production but not DNA decline.

Given the limited benefit of immunotherapeutic drugs used as single agents, the expectation is that combination therapy will be required to achieve hepatitis B cure. This combination will likely include DAA in combination with immunomodulatory drugs inducing complementary mechanisms of action to facilitate antiviral immunity in the liver. Perhaps the most promising immune combination so far is therapeutic vaccine administered together with checkpoint inhibitor PD-L1, which results in sustained decrease of HBsAg in the woodchuck model. Combining immune drugs with DAA that reduce HBsAg could be a potential avenue to restoring T cell functionality. In the AAV-HBV mouse model, the combination of TherVacB therapeutic vaccine with anti-HBV siRNA resulted in sustained reduction in HBsAg levels long after cessation of drug treatment. This approach is currently being pursued in the clinic. Finally, nucleic acid polymers acting as secretion inhibitors have clinically demonstrated strong HBsAg reduction when combined with TDF and pegIFN, resulting in a 35% rate of functional cure in all patients treated. Given the currently limited data showing benefit of combining two modalities, three agents may be needed to achieve higher levels of functional cure.

Principles of hepatitis E virus replication, persistence and antiviral strategies. Eike Steinmann, Ph.D., Ruhr University in Bochum, Bochum, Germany

Eike Steinmann's talk focused on hepatitis E virus (HEV). HEV is the causative agent of hepatitis E and the leading cause of acute viral hepatitis, affecting approximately 20 million individuals and resulting in 3.3 million symptomatic infections and 44,000–70,000 deaths per year. HEV is a zoonotic, single-stranded, positive-sense RNA virus of about 7 kb sub-classified into eight genotypes. The main transmission route to humans in Europe and the US is through consuming undercooked pork contaminated with HEV genotype 3. Other sources of infection include consumption of contaminated water, shellfish, crops, and other meat preparations. In Africa and large parts of Asia, the main mode of transmission is drinking water contaminated with genotypes 1 or 2. Although HEV is usually a self-limiting disease, immunocompromised individuals are at risk of developing chronic infection that rapidly progresses to fibrosis, cirrhosis, or even liver failure.

Current therapy options to treat hepatitis E are limited to the unspecific antivirals RBV and pegIFN. RBV leads to viral clearance in only 80% of chronically infected patients. However, RBV has not been evaluated in acutely infected patients and is contraindicated in pregnant women, a major high-risk group, emphasizing the urgency of developing new therapy options. The mechanism of action of RBV is complex and involves both direct antiviral effects and immune stimulation. In responders, RBV increases mutations in the viral genome, leading to amino acid changes. Although these mutations are not associated with RBV resistance, some of them increase viral fitness. Strains with increased fitness have been used to improve in vitro cultivation of HEV in hepatoma cells.

Both host and viral targets have been evaluated in vitro for future HEV therapies. The RNA polymerase inhibitor sofosbuvir, already approved to treat HCV infections, failed to inhibit HEV in the clinic. Currently, the only drug candidate with demonstrated in vivo anti-HEV effect is the natural product silvestrol, which acts as a host translation inhibitor. Derivatives of silvestrol are currently being evaluated in vitro.

CD40 agonists boost IFN-induced signalling pathway and subsequent anti-HBV response in vitro and in vivo. Antoine Alam, Ph.D., Evotec ID, Lyon, France

Antoine Alam discussed the continued need for improved therapeutics for CHB. The combination of CD40 agonism and type-I IFN stimulation (IFN-β, specifically) in inhibiting HBV infection was explored both in vitro and in vivo. CD40L boosts the anti-viral effects of IFN-β in HBV-infected primary human hepatocytes, leading to decreased HBeAg and pgRNA. This combination also increased the release of the IFN-responsive protein CXCL10, but not the inflammatory protein IL-8. The combination boosted other interferon stimulated genes, such as CXCL9, CXCL11, and ISG20, a key player in innate antiviral immunity. Furthermore, co-administering CD40L and IFN-β to AAV/HBV-infected mice led to significant and synergistic reduction of all viral parameters, including circulating HBV DNA, HBeAg, and HBsAg, as well as pgRNA and intra-hepatic HBV DNA. Importantly, ex vivo treatment of either human or murine whole blood cells with CD40L and IFN-β did not significantly induce inflammatory markers, such as IL-6 or TNF-α. Together, these results show that the combination of CD40L and IFN-β has potent anti-HBV activity in vitro and in vivo with minimal inflammation. Such a combination may have important therapeutic effect in CHB patients.

Efficient inhibition of hepatitis B virus cccDNA and pregenomic RNA by HBV ribonuclease h inhibitors during infection of HepG2-NTCP cells. Ranjit Chauhan, Ph.D., Saint Louis University, Saint Louis, Missouri, USA

Ranjit Chauhan discussed HBV and antiviral targeting of the viral ribonuclease H (RNaseH). Establishment of HBV cccDNA early after HBV infection through recircularization of viral replicative intermediates contributes to HBV persistence. RNaseH is a promising drug target, but how its inhibition impacts cccDNA formation is unknown. Three RNaseH inhibitors from different chemotypes, 1133 (N-hydroxypyridinedione, $EC_{50} = 0.11$ μM), 110 (hydroxytropolone $EC_{50} = 0.30$ μM), and 1073 (N-hydroxynapthyridinone, $EC_{50} = 1.5$ μM), efficacious against intracellular HBV DNA accumulation in inducible replication systems, were tested for effects on HBV product formation in infected HepG2- NTCP cells. Cells were infected for 12 h and compounds were added immediately following infection at concentrations of 0.05 μM, 0.5 μM, and 5 μM. Total intracellular HBV DNA, cccDNA, pgRNA, and total HBV RNA accumulation were evaluated 7 days post infection. The inhibition of total intracellular HBV DNA was dose responsive, with ~99% inhibition (compared to vehicle control) achieved by all compounds at 0.5 μM. All compounds inhibited cccDNA formation by 75–95% at 0.5 μM. Inhibition of cccDNA was reflected in suppression of pgRNA levels by >90% (by 1133 and 110) and >50% (by 1073). Similar inhibition was detected for all HBV RNAs using primers targeting the HBx region. In conclusion, HBV RNaseH inhibitors can efficiently suppress cccDNA formation in vitro. Viral suppression was more pronounced than predicted by EC_{50}s in stably transfected cells, presumably due to suppression of cccDNA amplification. These data support progression of RNaseH inhibitors as therapeutic candidates for the treatment of CHB.

Farnesoid X receptor alpha ligands inhibit hepatitis delta virus replication and propagation in physiologic cell culture model. Julie Lucifora, Ph.D., INSERM, Ciri, Lyon, France

Julie Lucifora spoke about HDV, a satellite of HBV. Both HBV and HDV use the human sodium taurocholate co-transporting polypeptide (hNTCP), the main transporter of bile acids (BA) in the liver, to enter hepatocytes. Links between BA and HBV infection are not limited to the entry step. Indeed, the farnesoid X receptor alpha (FXR), the nuclear receptor of BA, is a proviral factor for HBV and FXR ligands act as inhibitors of HBV replication. The putative links between BA metabolism, FXR, and HDV replication have not been explored. In HepaRG and primary human hepatocytes co- or super-infected with HDV/HBV, treatment with FXR ligands like GW4064, ECDCA, or tropifexor significantly decreased the levels of total intracellular HDV RNAs by ~50%. The effect was reversed in FXR loss-of-function experiments, confirming the specificity of action. Immunofluorescent staining and western blot analyses of infected cells showed that FXR ligands also modestly decreased the amount of intracellular delta antigens. The effect on viral progeny was very strong, with >98% loss of infectivity, as assessed by reinfection of Huh7.5-hNTCP cells. FXR ligands potently inhibit HDV replication and propagation in vitro, independently of their effect on HBV. The antiviral effect was far superior to that of IFNα, the current standard of care for chronic HDV patients. Although the precise mechanism of HDV inhibition associated with FXR agonists has yet to be elucidated, FXR appears to represent an attractive target for HDV antiviral therapy.

Session 9: technology and Antonín Holý Memorial Award

The last session on Wednesday, March 25th, provided the opportunity to get updated on some new and interesting technologies that are just starting to be applied to antiviral research and will likely have an increasing impact in the near future. The session was chaired by Dr. Jennifer Moffat and Dr. Andrea Brancale, and included three outstanding keynote presentations by Dr. Matthew Disney, Dr. Chris Meier, and Dr. Christiane Wobus. These invited talks were preceded by the Presentation of the Antonín Holý Memorial Award Lecture, delivered by this year's Award recipient Dr. Eddy Arnold. The presentations were all pre-recorded; however, a most interesting and lively live Q&A with all speakers concluded the session.

The Holý Award recipient lecture: the long and winding road: 35 years of HIV reverse transcriptase structure, mechanism, and successful anti-AIDS drug design. Eddy Arnold, Ph.D., Rutgers University, New Brunswick, New Jersey, USA

Eddy Arnold gave a very personal account of his remarkable scientific career. Eddy is well known for his incredible contributions to HIV research, especially in the fields of structural biology and drug design. His talk showed that he was a true pioneer in understanding on mechanism by which non-nucleoside reverse transcriptase inhibitors (NNRTI) bind to the reverse transcriptase of HIV-1. These studies were a challenging endeavor, partly because the enzyme is very flexible and adopts multiple conformations; the NNRTI binding pocket is not even visible in the *apo* reverse transcriptase (RT) structures. With his skills and knowledge, Eddy contributed significantly to the discovery of two FDA-approved NNRTI drugs (etravirine and rilpivirine) and development of six licensed medicines. In his talk, Eddy presented his scientific achievements as a journey. More importantly, he fondly acknowledged the remarkable people he met along his path. His talk was very warm and personal, loaded with anecdotes that made the story about the discovery and development of NNRTIs compelling and real. Indeed, during the Q&A session, he also answered a question about his interactions with Dr Paul Janssen, and his words were still full of admiration and gratitude, recognizing Dr Janssen as a leader and innovator in the field. A modest, yet outstanding, scientist, Eddy proved to be a very worthy Holý awardee.

Sequence-based design of small molecules targeting RNA. Matt Disney, Ph.D., Scripps Institute, La Jolla, California, USA

Matt Disney began the presentations on new technologies on antiviral research. His talk discussed the development of new molecules and strategies to target viral RNA, revealing the potential of targeting RNA in antiviral drug discovery. This approach could rival or, more likely, complement, the standard antiviral approaches that target viral proteins. Matt described two strategies on which his group is working. One strategy is to understand the three-dimensional structure of viral RNA using available sequences and then using this knowledge to identify compounds that could bind to specific conserved structural motifs. In this context, he discussed his work on identifying new compounds that bind SARS-CoV-2 RNA. The second strategy focusses on recruiting ribonuclease enzymes to facilitate the processing of viral RNAs. This intriguing approach is in some ways similar to PROTAC, which is becoming increasingly popular in drug discovery. During the Q&A session, Matt answered a few questions about selectivity and resistance barrier of RNA binding compounds. He also highlighted that preprint servers are becoming an increasingly important way to share information efficiently and quickly, and emphasized the importance of these servers to his group's work.

Design of nucleotide prodrugs for antiviral chemotherapy—the TriPPPro-approach. Chris Meier, Ph.D., Universität Hamburg, Hamburg, Germany

Nucleosides are the most prevalent class of antiviral drugs, generally active as triphosphate analogs. However, this highly charged form does not cross biological membranes, and thus cannot be administered as a drug as such. A prodrug approach (the ProTide strategy) was successfully applied to deliver nucleoside monophosphate into cells in recent years, but phosphorylation to achieve the active triphosphate form still requires the participation of cellular enzymes, which discriminate against certain types of modified nucleosides. With the new approach developed by **Chris Meier**, the active triphosphate form is cleverly masked and thus able to cross cellular membranes, and is then released inside the cell to exert its activity. The new approach, TriPPPro, which is currently state-of-the art, has the potential to open a new era in nucleoside drug discovery. Chris discussed this point during the Q&A, and although it is difficult to predict whether this approach will be clinically successfully in the end, it is already showing the maturity to be developed towards clinical applications in the near future.

Investigating enterotropic virus infections in human intestinal organoids. Christiane Wobus, Ph.D., University of Michigan, Ann Arbor

Christiane Wobus discussed her work on organoids and 3D cultures in drug discovery. Organoids are becoming an important tool available for drug discovery and

development. They are often considered to be more representative of physiological conditions than standard two-dimensional cell culture, and more accurately mimic in vivo results. Although these cultures may appear cumbersome and with limited reproducibility, Christiane discussed how flexible they truly are and showed that they can be generated from patient-derived cells. A very interesting work described by Christiane centers around enteroids. She discussed the ability to create these "mini-guts" with an inner cavity and described how useful they are in studying viruses that infect the GI tract, including SARS-CoV-2 (50% of COVID-19 patients have GI tract symptoms). During the Q&A, Christiane gave her view of the future of more widespread organoid use in antiviral drug discovery. This approach is now also establishing itself as an extremely useful tool in other research fields.

Overall, this was a truly exciting and inspirational session, which presented a view of some fascinating new technologies that will most likely become familiar in the near future. The audience was inspired to continuously evaluate these and similar new technologies for their potential applications in antiviral drug discovery and development.

Session 9: other respiratory viruses

Emerging deadly viruses and their glycoproteins—From infection to vaccines and antivirals. Hector Aguilar-Carreno, Ph.D., Cornell University, Ithaca, New York, USA

The COVID-19 pandemic has emphasized the ongoing need to study emerging zoonotic pathogens. **Hector Aguilar-Carreno** and his laboratory have spent over two decades studying the glycoproteins of such pathogens, including Nipah, Hendra, Ebola, and influenza viruses, and, more recently, SARS-CoV-2. Utilizing both classical and molecular virological methods, his group's work exemplifies the potential translation of basic science to the future development of novel vaccines and therapeutics against these viruses.

Hector discussed the development and evaluation of a multi-valent VSV vaccine concurrently pseudotyped with the viral entry glycoproteins of Nipah, Hendra, and Ebola viruses and showed that this approach provides complete protection against lethal challenge with each virus in Syrian hamsters. The viability of this platform to address infections with ecological and potentially epidemiological overlap can be applied to generating potential pan-coronavirus vaccines, an approach that Hector's group is currently pursuing.

Through the discovery and characterization of a series of membrane-intercalating compounds, Hector's group has developed a compound (XM-01) as an inactivation agent that preserves viral glycoproteins in their native conformations. Mice immunized with XM-01-inactivated influenza showed improved survival and decreased morbidity from viral challenge when compared to mice immunized with influenza inactivated by formalin fixation. Moreover, sera from mice immunized with XM-01-inactivated influenza virus developed comparably more potent anti-HA and anti-NA neutralizing antibodies.

Hector's group has also recently established multiple animal models for studying coronaviral infections, including K18 hACE2 mice and Syrian hamsters for SARS-CoV-2 research, and is currently investigating an intranasally administered TMPRSS2 protease inhibitor that significantly protects K18 ACE2 mice from SARS-CoV-2-induced morbidity and mortality.

Remdesivir treatment for emerging virus infections. Emmie de Wit, Ph.D., NIAID, Hamilton, Montana, USA

Emmie de Wit presented work with colleagues evaluating remdesivir in NHP models of Nipah virus, MERS-CoV, and SARS-CoV-2 infection. Remdesivir is an adenosine nucleotide prodrug approved for treatment of COVID-19. The prodrug is metabolized to the active triphosphate form used as a substrate by the viral polymerase. Incorporation of the nucleotide analog into the viral RNA during replication inhibits the viral polymerase, leading to reduced virus replication. Remdesivir has broad-spectrum activity and is active in cell culture against filoviruses, coronaviruses, and paramyxoviruses. African green monkeys ($n = 4$ per group) were inoculated with 2×10^5 $TCID_{50}$ of Nipah virus by intranasal and intratracheal routes. Remdesivir (10 mg/kg) or placebo (vehicle) was administered once daily by IV bolus for 12 days starting at 24 h post inoculation. Placebo-treated animals exhibited signs of severe disease and were euthanized by day 8 when they reached signs requiring humane euthanasia. Signs of disease in animals treated with remdesivir were notably less severe compared to placebo treated animals. Viral loads in the upper respiratory tract (nose and throat) were similar in all treatment groups, but no viremia or severe lung disease was observed in animals treated with remdesivir; all remdesivir-treated animals survived to day 92 of the study. A single remdesivir-treated animal had histopathological evidence of meningoencephalitis but no neurological signs were observed.

Remdesivir was also evaluated in a rhesus macaque model of MERS-CoV infection. Animals ($n = 6$ per group) were inoculated with 1×10^6 $TCID_{50}$ of MERS-CoV-2 by intranasal, intratracheal, or ocular routes. Animals were treated with 5 mg/kg of remdesivir or placebo by IV administration either one day prior to inoculation or 12 h post inoculation, followed by daily treatments on days 1–6. Animals were euthanized on day 6 post inoculation and

antiviral efficacy was measured. Remdesivir treatment reduced clinical signs of disease, viral load in the lungs, and lung lesions measured by histopathology compared to placebo. The antiviral effects were more pronounced in animals treated 24 h prior to inoculation than in animals treated 12 h post inoculation.

A rhesus macaque model of SARS-CoV-2 infection was developed that resembled mild human disease with measurable pulmonary infiltrates observed on radiographs and by histopathology. Animals inoculated with SARS-CoV-2 by intranasal, intratracheal, oral, or ocular routes exhibited high levels of viral shedding in the nose and throat and intermediate detection of virus in rectal swabs. No viral RNA was detectable in urogenital swabs or in blood and urine. Viral loads peaked 1 day post inoculation and infectious virus was cleared more quickly than viral RNA from the lungs. Animals (n = 6 per group) were treated with placebo or remdesivir starting at 10 mg/kg 12 h post inoculation followed by 6 consecutive daily treatments of 5 mg/kg. Animals were euthanized on day 7 post inoculation. Remdesivir did not impact shedding of virus in the nose or throat or in rectal swabs compared to placebo-treated animals. However, relative to placebo-treated animals, remdesivir treatment reduced clinical disease, viral lung pathology, and viral titers in bronchoalveolar lavages (BAL) 12 h after the initial treatment and in lung tissue on day 7 post inoculation. These data support continued evaluation of remdesivir for treating paramyxovirus and coronavirus infections.

The ABCs of rhinovirus infections and asthma. James Gern, M.D., University of Wisconsin, Madison, Wisconsin, USA

James Gern presented work with colleagues to set the stage for developing vaccines against rhinovirus. Rhinovirus infections are highly associated with wheezing illness in children over 1 year of age and are an important risk factor for developing asthma later in life. Rhinoviruses are also an important trigger exacerbating asthma in adults and children. Three main genotypes of rhinovirus exist, RV-A, RV-B, and RV-C, with approximately 170 associated types that co-circulate in the human population causing a spectrum of respiratory disease from asymptomatic infection or mild upper respiratory disease to pneumonia. RV-A and RV-C are most often associated with clinical disease. The antigenic diversity associated with rhinoviruses is a primary reason rhinovirus infections are maintained in the human population causing seasonal infections year after year. The antigenic diversity of rhinovirus strains presents a challenge for developing effective vaccines to prevent or reduce disease associated with rhinovirus infection.

Longitudinal data from the Childhood Origins of Asthma (COAST) birth cohort study were analysed to determine the relationships between age and RV-C infections. Neutralizing antibodies specific for RV-A and RV-C were determined using a novel PCR-based assay. Data were pooled from 14 study cohorts in the United States, Finland, and Australia, and mixed-effects logistic regression was used to identify factors related to the proportion of RV-C versus RV-A detection.

RV-A and RV-C infections were common in infancy, whereas RV-C was detected much less often than RV-A in older children. The prevalence of neutralizing antibodies to RV-A or RV-C types was low in children 2 years of age and increased through the teen years. At each age, RV-C seropositivity was 3–5 times more prevalent. The ratio of RV-C to RV-A titers during illnesses was significantly related to age, CDHR3 genotype (putative RV-C receptor and asthma susceptibility allele), and wheezing illnesses. These data suggest the RV-C may be more immunogenic than RV-A causing more disease in early life but less disease as children age due to development of neutralizing antibodies. Identifying rhinovirus types associated with symptomatic disease and recognizing that RV-C neutralizing antibodies are protective reduces the complexity of antigens required for development of a polyvalent vaccine.

Roles of PNKP and CDK1 in Zika virus replication and pathogenesis. Malgorzata (Gosia) Rychlowska, Ph.D., Cornell University, Ithaca, New York, USA

Malgorzata (Gosia) Rychlowska described the pathogenicity of Zika virus to neuronal cell progenitors. Zika virus is a mosquito-borne flavivirus that can be both sexually and vertically transmitted in humans, with the latter sometimes resulting in neonatal microcephaly due to Zika virus-induced DNA damage in human neural progenitor cells. Given that rare mutations in the DNA damage repair protein polynucleotide kinase (PNKP) also results in microcephaly, Gosia and colleagues sought to evaluate whether Zika virus infection affected PNKP. A PNKP inhibitor showed dose-dependent inhibition of Zika virus infection. Through fluorescence microscopy, Zika virus infection induced cytoplasmic co-localization of PNKP with Zika virus NS1. Interestingly, a mutant with cytoplasmic PNKP phenotype results in microcephaly. Zika virus induced mitotic abnormalities consistent with the morphological hallmarks of mitotic catastrophe in neural progenitor cells, and a cell cycle inhibitor blocking CDK1 (roscovitine) was shown to inhibit Zika virus replication, likely by inhibiting formation of replication complexes from the endoplasmic reticulum. Since CDK1 colocalized with Zika virus NS1 by fluorescence microscopy, the accumulation of CDK1 and the CDK1 activator Cyclin A was tested and the complexes were immunoprecipitated from infected

cells to assess their biochemical activity by kinase assays. The results from this study indicate that Zika virus likely induces CDK1 to form replication complexes while displacing PNKP into the cytoplasm, thereby resulting in mitotic catastrophe.

Plant lectin urtica dioica agglutinins (UDA) is a potential inhibitor against rabies virus in a muscle model. Xinyu Wang, M.S., Rega Institute, Laboratory of Virology and Chemotherapy, KU Leuven, Leuven, Belgium

Xinyu Wang focused on strategies for treating rabies virus. Rabies virus is transmitted to humans through the bite of an infected animal; left untreated, rabies is almost uniformly fatal. Current treatments involve early administration of rabies immunoglobulin and vaccination. While these treatments are effective, they are expensive and have limited availability. Xinyu and colleagues screened a lectin library to identify inhibitors of rabies virus infection in cell culture. Two lectins, UDA and BanLec, were identified as inhibitors of rabies virus replication with EC_{50} values of 8.2 and 7.2 μg/mL, respectively. Time of addition studies demonstrated that UDA binds to the cell surface to block virus entry. UDA pre-treatment reduced viral yield in cell culture 5-fold. A model using isolated pig muscle explant was developed to measure rabies virus replication. Treating pig muscle tissues with UDA ($3 \times EC_{50}$ value) reduced rabies virus replication over 100-fold relative to vehicle treatment. Replication was quantified using a rabies reporter virus expressing mCherry protein and by immunohistochemical staining for the rabies virus N protein. These data suggest that lectins could be an alternative treatment for rabies virus infection.

Arbovirus infectivity is significantly reduced by components of the bacterial cell wall. Lana Langendries, M.S., Rega Institute for Medical Research at KU Leuven, Leuven, Belgium

While transmission of arboviruses (e.g., Zika, dengue, yellow fever, and chikungunya viruses) through mosquito bites at the skin has been well characterized, little is known about the interactions of arboviruses with skin microbiota present at the site of infection. To investigate this question, **Lana Langendries** studied the effects of bacterial cell wall components on the infectivity of such arboviruses. Incubating alphaviruses (chikungunya and Semliki Forest viruses) with lipopolysaccharides (LPS) or lipoteichoic acids and peptidoglycans of Gram-positive bacteria significantly reduced the infectivity of these viruses in vitro. This was not due to cell-dependent effects, as preincubating cells with LPS prior to infection did not affect viral infectivity. Furthermore, treating cells with Toll-like receptor 4 inhibitor did not ablate the LPS-dependent inhibition of viral infectivity. A virucidal assay involving incubating LPS-treated viruses with or without RNAse showed that LPS incubation rendered alphavirus genomes more susceptible to RNase degradation, suggesting that LPS compromises the structural integrity of the viral envelope. Transmission electron micrographs of Semliki Forest virus incubated with LPS showed progressive changes in virion morphology, which correlated with decreasing viral infectivity, thus further supporting the case for a virucidal effect of bacterial LPS against arboviruses.

Concluding remarks

ISAR actively addressed the challenges posed by a viral pandemic by supporting antiviral and vaccine discovery and the research and development community through sharing of curated pre-prints and other information early in the pandemic. These and other efforts, including ICAR 2021, supported the lively and timely exchange of information about researchers, developers and other stakeholders involved in curtailing the effects of the pandemic. Although the virtual format of the 2021 annual meeting prevented some of the exchanges that occur at live ICAR meetings, overall, the goals of the annual meeting were achieved and the event was extremely successful at supporting the Society's mission.

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